X-RAY ANALYSIS OF SOME HUNTERBURNINE AND SANTONIN DERIVATIVES

BEING A THESIS FOR THE DEGREE OF

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SUBMITTED BY

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PREFACE.

I wish to express my thanks to Dr. G.A. Sim and Professor J. Monteath Robertson for their advice and direction throughout this period of research.

The extensive calculations were performed on the Glasgow University DEUCE computer. I am indebted to those authors who made their programmes for DEUCE available to me, in particular Dr. J.S. Rollett and Dr. J.G. Sime.

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SUMMARY

X-ray studies have been carried out on crystals of heavy-atom derivatives of naturally-occurring organic compounds and related photo-irradiation products. Four structures have been successfully solved: the alkaloid hunterburnine, the sesquiterpenoid santonin and its photo-irradiation product isophoto-α-santonic lactone, and the colouring material ergoflavin. Attempts to solve the structure of toxisterol A_2, a photo-irradiation product of the steroid ergosterol, have proved fruitless.

The elucidation of the structure of hunterburnine resulted from a straightforward application of the heavy atom technique to hunterburnine methiodide and the complete structure was assigned with certainty from the fifth three-dimensional Fourier synthesis. Subsequent refinement reduced the discrepancy, R, to 15.4%. Hunterburnine represents a new class of indole alkaloid.

The analysis of bromodihydroisophoto-α-santonic lactone acetate was undertaken to define the stereochemistry of the parent molecule. The analysis was hindered in the early stages by spurious symmetry which was eventually overcome with the aid of chemical knowledge of the structure. The structure has been refined and

the final value of R is 12.9%. The analysis has established the relative stereochemistry of isophoto-α-santonic lactone.

The configuration of the methyl group on the lactone ring of isophoto-α-santonic lactone is opposite to that generally accepted, implying that the assignation of the stereochemistry of the corresponding methyl group in santonin itself was in error. Hence an analysis of the heavy-atom derivative 2-bromo-α-santonin was undertaken. In spite of spurious symmetry, the structure was solved and refined to give a discrepancy of 15.2%. The analysis has confirmed the revised configuration of the contraversial methyl group in santonin.

Inter- and intra-molecular dimensions in these three structures are in reasonable agreement with accepted values.

The structure and stereochemistry of tetramethyl ergoflavin di-p-iodobenzoate have been established and refinement of the structure is in progress. Molecular dimensions have not been discussed, but the gross molecular structure is in agreement with chemical evidence with the exception of the assignment of hydroxyl and phenolic groupings. This project was studied in conjunction with A.T. McPhail and J.V. Silverton.

The final chapter of this thesis describes an attempt to solve the structure of toxisterol A_2 4-iodo-3-nitrobenzoate which crystallises in the monoclinic system with space group $P2_1$. The heavy-atom positions have been located, but it has not been possible to allocate additional atomic sites from three-dimensional Fourier maps or a three-dimensional superimposed Patterson synthesis.

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PART I.

SOME METHODS OF CRYSTAL STRUCTURE ANALYSIS

1. (1) INTRODUCTION.

In 1912, Friedrich and Knipping, at the instigation of von Laue, took the first x-ray photograph. It was soon realised that the diffracted x-ray beams could be used to give the image of the atomic arrangement causing the scattering. Some of the theory relating the atomic pattern to the intensity of the diffracted beams and some techniques employed in elucidating the atomic arrangement are reviewed in the course of this chapter.

Two major factors have restrained the development of the x-ray method of structure determination. Firstly, part of the information, the phases, required to give a representation of the atoms in a crystal is lost when the experiment of recording intensities of the diffracted beams is made. In this thesis, the "heavy-atom" method has been employed to give an initial set of approximate phases which have been modified by iterations of structure factor and Fourier calculations, including successively more and more atoms until the structure determination was complete.

Secondly, there is the practical difficulty of the immense calculations which have to be performed in the course of an analysis. Before the advent of high-speed computing facilities, this factor severely limited the size of the molecule studied and the degrees of accuracy and

completeness achieved.

Today, the results from x-ray analysis can be divided into two categories. Firstly, they can be used to give bond lengths and bond angles in the molecule under study and, also, distances between atoms in neighbouring molecules. Such results are essential if advances are to be made in the theory of chemical bonding.

Secondly, they can provide a complete picture of hitherto unknown complex molecules. This thesis is concerned primarily with results of the second category and is an investigation of the structure and stereochemistry of some naturally occurring organic compounds and related photo-irradiation products.

1. (2) THE LAUE EQUATIONS.

A crystal consists of a repetitive three-dimensional array of atoms, molecules or ions. If an arbitrary origin is defined, then it will be possible to find further points in space which have an environment identical to that of the origin. These points describe a lattice which can be defined in terms of three non-coplanar vectors $\underline{a}$, $\underline{b}$ and $\underline{c}$. These vectors bound a volume which is called the unit cell. The lattice is important as it determines completely the conditions for diffraction without consideration of any details of the

structure.

If some scattering material, say an electron, is positioned at each lattice point, the positions of these electrons can be defined by a set of vectors $\underline{r}$, such that

$$\underline{r} = u\underline{a} + v\underline{b} + w\underline{c}$$

where u, v and w are integers, and $\underline{a}$, $\underline{b}$ and $\underline{c}$ are the vectors defined above. A and B in Fig. 1 are two such lattice points.

A beam of x-rays of wavelength λ is now allowed to fall on the lattice in a direction defined by a vector $\underline{s}_o$ with magnitude $1/\lambda$. Consideration of the phase differences between the waves scattered in any particular direction generates the Laue equations.

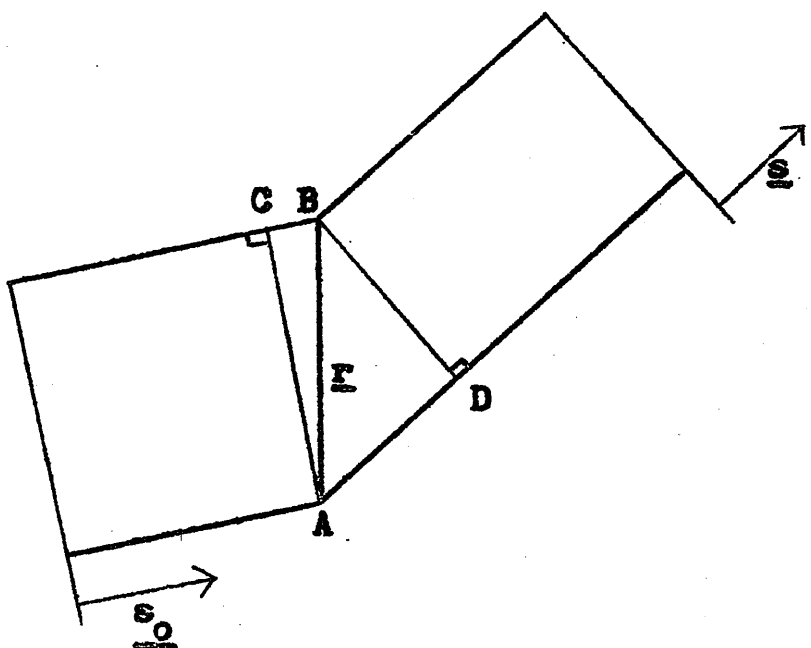


Fig. 1.

The path difference between the scattered waves from the two lattice points in a direction defined by the vector $\underline{s}$ equal in magnitude to $\underline{s}_0$ is given by

$$AD - CB = \lambda(\underline{r}.\underline{s} - \underline{r}.\underline{s}_0) = \lambda\,\underline{r}.\underline{R}$$

where $\underline{R} = \underline{s} - \underline{s}_0$.

The condition for the waves scattered at A and B to be in phase is that the path difference $\underline{r}.\underline{R}$ must be an integer, i.e.

$$(u\underline{a} + v\underline{b} + w\underline{c}).\underline{R} = \text{integer}.$$

Since this must be true when u, v and w change by integral values, and since u, v and w are already integral, it follows that

$$\underline{a}.\underline{R} = h$$

$$\underline{b}.\underline{R} = k$$

$$\underline{c}.\underline{R} = \ell$$

where h, k and ℓ are integers.

These equations are known as Laue's equations.

1. (3) BRAGG'S LAW.

The form of the Laue equations makes them inconvenient for interpretation of experimental results. Bragg identified the relationship between the integers h, k and ℓ, and the Miller indices, and reduced the problem to one

dimension.

The three Laue equations may be rewritten in the form

$$\underline{a}/h \cdot \underline{R} = 1$$

$$\underline{b}/k \cdot \underline{R} = 1$$

$$\underline{c}/l \cdot \underline{R} = 1$$

From the first two of these equations, the following relationship is obtained

$$(\underline{a}/h - \underline{b}/k) \cdot \underline{R} = 0$$

Thus the vector $\underline{R}$ is at right angles to the vector $\underline{a}/h - \underline{b}/k$; in Fig. 2, $\underline{R}$ is perpendicular to PQ. In a similar fashion it can be shown that $\underline{R}$ is perpendicular to QR and RP in turn.

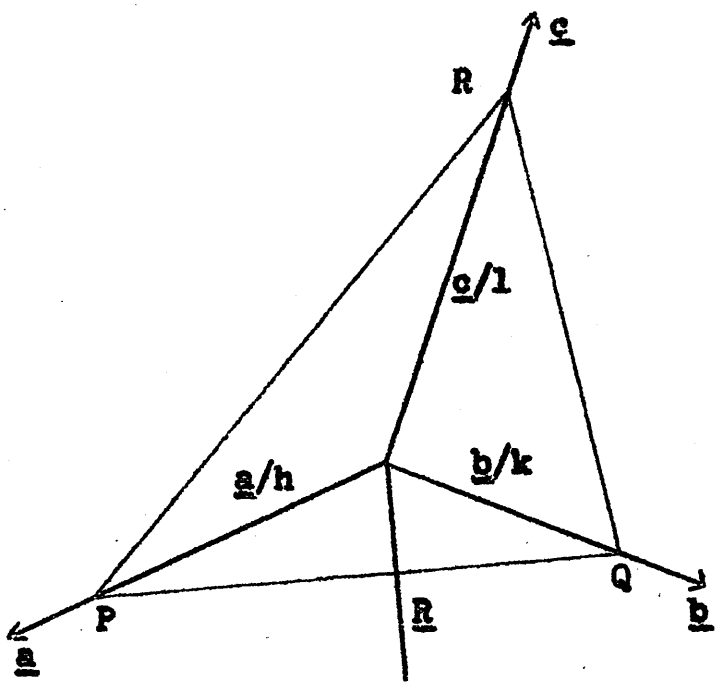


Fig. 2.

Hence $\underline{R}$ is perpendicular to the plane PQR which has intercepts $(\underline{a}/h, \underline{b}/k, \underline{c}/\ell)$ on the unit cell axes. Thus $\underline{R}$ is in the direction of the normal to the plane of Miller indices $(h\ k\ \ell)$.

In Fig. 3, $\underline{s}_o$ makes an angle θ with the plane, and since $|\underline{s}_o| = |\underline{s}|$ by definition, $\underline{R}$ must be the bisector of the incident and diffracted beams. Thus $\underline{s}$ must also be inclined at an angle θ, and so diffraction may be regarded as a reflection from the lattice plane with Miller indices $(h\ k\ \ell)$.

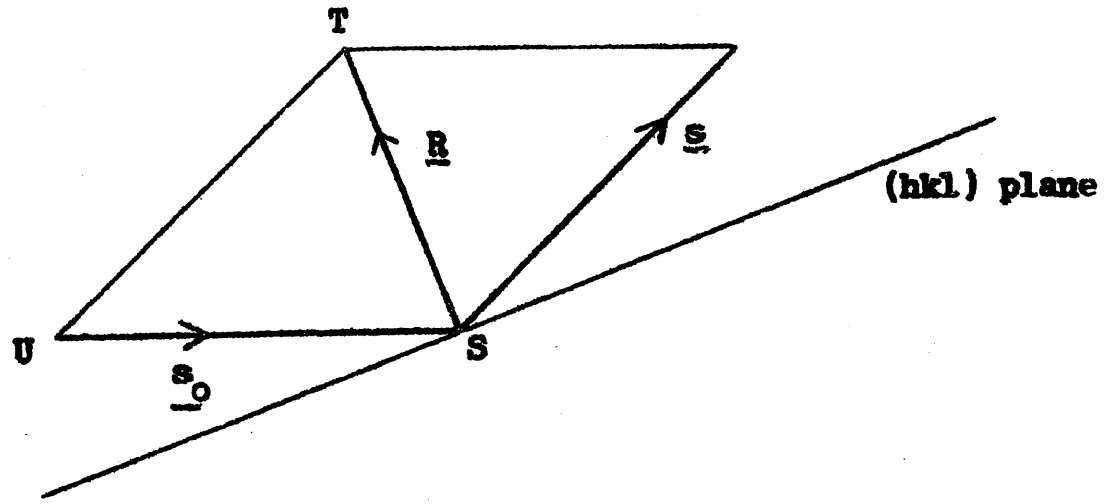


Fig. 3

If US represents the vector $\underline{s}_o$, then

$$|R| = ST = 2US\cos(90^\circ - \theta)$$
$$= 2 \sin\theta/\lambda$$

Now d, the distance from the origin to the plane PQR, is the projection of the vector $\underline{a}/h$, $\underline{b}/k$ or $\underline{c}/\ell$ on the vector $\underline{R}$

i.e. $$d = \underline{a}\ (h.\underline{R})/|\underline{R}|$$
$$= \lambda/2 \sin\theta,$$

since $a/h.\underline{R} = 1$ by Laue's equations

i.e. $$\lambda = 2\ d \sin\theta$$

This equation is known as Bragg's Law.

1. (4) THE STRUCTURE FACTOR.

In the previous sections, the scattering unit was assumed to be an electron positioned at a lattice point. In reality, the electrons surrounding the atomic nucleus scatter the x-rays. The distribution of electrons round each atom, known from atomic structure theory, enables the evaluation of the scattering factor (f_o) to be made; it is the ratio of the amplitude scattered by the total number of electrons in the atom (Z, say) in a given direction to the amplitude scattered by a single electron in the same direction. In atoms, electrons occupy a finite volume (the atomic volume), and phase differences will arise between rays scattered by the various electrons in this volume. These phase differences are dependent on the

value of θ, the angle of diffraction given in Bragg's Law. If θ is small, the phase differences are small, and f_o approaches the value Z. As the angle of diffraction increases, the value of f_o falls. The curve of f_o against $\sin\theta/\lambda$ is the scattering factor curve.

Consideration must now be given to the mode of combination of the scattered rays from each atom. A crystal with N atoms in the unit cell may be imagined to consist of N interpenetrating lattices, with all the lattice points occupied by atoms. Each lattice will obey the Laue and Bragg conditions, but in general the waves scattered by the N lattices will be out of phase.

The mean position of the j^{th} atom, situated at the point (x_j, y_j, z_j) where x_j, y_j and z_j are fractions of the unit cell vectors, may be represented by $\underline{r}_j$, where

$$\underline{r}_j = x_j\underline{a} + y_j\underline{b} + z_j\underline{c}$$

The path difference between the waves scattered by the j^{th} atom and an atom at the origin is $\lambda\underline{r}_j.\ \underline{R}$ and the corresponding phase difference is $2\pi\,\underline{r}_j.\ \underline{R}$. Combining these phase differences for all the atoms in the unit cell, the expression for the complete wave scattered in the crystal would be

$$F = \sum_{j=1}^{N} f_j \exp.2\pi i \underline{r}_j.\underline{R}$$

$$= \sum_{j=1}^{N} f_j \exp.2\pi i (x_j \underline{a}.\underline{R} + y_j \underline{b}.\underline{R} + z_j \underline{c}.\underline{R})$$

$$\therefore F(hk\ell) = \sum_{j=1}^{N} f_j \exp.2\pi i (hx_j + ky_j + \ell z_j)$$

where f_j is the scattering factor and $F(hk\ell)$ the structure factor.

$F(hk\ell)$ is a complex quantity and may be expressed in terms of its real and imaginary components

$$F(hk\ell) = A(hk\ell) + i\,B(hk\ell)$$

Since $\exp.i\phi = \cos\phi + i\sin\phi$

$$F(hk\ell) = \sum_{j=1}^{N} f_j \cos 2\pi (hx_j + ky_j + \ell z_j) + i \sum_{j=1}^{N} f_j \sin 2\pi (hx_j + ky_j + \ell z_j)$$

Equating the real and imaginary parts of the two expressions for $F(hk\ell)$ gives

$$A = \sum_{j=1}^{N} f_j \cos 2\pi (hx_j + ky_j + \ell z_j)$$

$$B = \sum_{j=1}^{N} f_j \sin 2\pi (hx_j + ky_j + \ell z_j)$$

The modulus of the structure factor, $|F(hk\ell)|$, is called the structure amplitude and is calculated from

$$|F(hk\ell)| = \sqrt{(A^2 + B^2)}.$$

The phase constant, α, is evaluated from

$$\alpha(h\ k\ \ell) = \tan^{-1} B/A.$$

An alternative, more general, approach to the structure factor expression is to consider each element of volume of the unit cell separately. If $\rho(xyz)$ is the density of scattering material at any point x, y, z, in the unit cell, the number of electrons in the volume element dxdydz will be $\rho(xyz)dxdydz$. For the general case, where the crystal axes, a, b and c are inclined at any angles, the number of electrons in the volume element will be $\rho(xyz)\frac{V}{abc}dxdydz$, where V is the volume of the unit cell. The structure factor expression then becomes

$$F(hk\ell) = \frac{V}{abc}\int_{ooo}^{abc}\int\int\rho(xyz)\exp.2\pi i(hx/a + ky/b + \ell z/c)dxdyds.$$

1. (5) TEMPERATURE FACTOR.

At all temperatures, atoms have a finite amplitude of oscillation. The thermal motion causes the atoms to scatter slightly out of phase, and scattering factors are reduced by an amount which increases with the diffraction angle θ. If the atomic scattering factor discussed in the previous section has symbol f_o, then the function f which is used in practice is given by

$$f = f_o \exp.(-B \sin^2\theta/\lambda^2)$$

where B is a constant called the temperature factor related to the mean-square amplitude of vibration ($\bar{\mu}^2$) by the

relationship $B = 8\pi^2\overline{u}^2$. A theoretical treatment of the subject and methods of evaluating B from fundamental constants and heat capacity data have been given by Debye. In general, the thermal displacement will not be isotropic, but will vary in different directions, and should be characterised by an ellipsoidal distribution. The asymmetric temperature factor assumed to describe this system (Cruickshank, 1956) is

$$B = \exp.(b_{11}h^2 + b_{12}hk + b_{13}h\ell + b_{22}k^2 + b_{23}k\ell + b_{33}\ell^2).$$

1. (6) FOURIER SERIES.

The electron density of a crystal is periodic in three dimensions and can therefore be represented by the sum of a suitable Fourier series

$$\rho(xyz) = \sum_{p=-\infty}^{+\infty}\sum_{q}\sum_{r} A(pqr)\exp.2\pi i(px/a + qy/b + rz/c)$$

where p, q and r are integers, and A(pqr) the Fourier coefficient of the general term.

This equation can be substituted in the general expression for the structure factor to give

$$F(hk\ell) = \frac{V}{abc}\iiint_{000}^{abc}\left[\sum_{p=-\infty}^{+\infty}\sum_{q}\sum_{r} A(pqr)\exp.2\pi i(px/a + qy/b + rz/c)\right] \exp.\, 2\pi i(hx/a + ky/b + \ell z/c)dxdydz.$$

$$= \frac{V}{abc}\iiint_{000}^{abc}\sum_{p=-\infty}^{+\infty}\sum_{q}\sum_{r} A(pqr)\exp.2\pi i\left[(p + h)x/a + (q + k)y/b + (r + \ell)z/c\right]dxdydz.$$

This integral has the value zero unless

$$h = -p, \quad k = -q, \quad \ell = -r$$

$$\text{when } F(hk\ell) = \frac{V}{abc}\iiint_{ooo}^{abc} A(\bar{h}\bar{k}\bar{\ell}) \exp. 0 \, dxdydz.$$

$$= V A(\bar{h}\bar{k}\bar{\ell})$$

$$\text{i.e. } A(\bar{h}\bar{k}\bar{\ell}) = F(hk\ell)/V$$

The three-dimensional Fourier series can now be written

$$\rho(xyz) = \frac{1}{V}\sum_{h=-\infty}^{+\infty}\sum_{k}\sum_{\ell} F(hk\ell) \exp. -2\pi i(hx/a + ky/b + \ell z/c)$$

The zero term of the series is a constant which may be obtained by integrating the general structure factor expression (see 1. (4)) over the whole of the unit cell.

$$F(000) = \frac{V}{abc}\iiint_{ooo}^{abc} \rho(xyz)dxdydz = Z$$

where Z is the total number of electrons in the unit cell.

1. (7) THE INTENSITY OF AN X-RAY REFLECTION.

Calculation of the electron density distribution in the unit cell requires a knowledge of structure factor values. These are not directly measurable, but by employing the concept of "integrated reflection", a relationship between intensity and structure amplitude can be derived.

If the crystal is rotated through the reflecting position with angular velocity, ω, and the total reflected energy is $E(hk\ell)$, then the integrated reflection is the ratio, $E(hk\ell)\omega/I_o$, given by

$$\frac{E(hk\ell)\omega}{I_0} = N^2\lambda^3\frac{e^4}{m^2c^4}LP|F(hk\ell)|^2dV$$

where dV is a small volume of crystal, N is the number of unit cells per unit volume, e^4/m^2c^4 arises from the expression for scattering due to a single electron, and I_0 is the intensity of the incident beam. L, the Lorentz factor, accounts for the motion of the crystal with respect to the beam, and P is the polarisation factor which makes the necessary allowance for the random polarisation of the incident beam.

The expressions for L and P depend on the kind of photograph being taken; for a moving-film-Weissenberg photograph $L = {}^1/\sin 2\theta$ and $P = (1 + \cos^2 2\theta)/2$.

This formula is based on the assumption of a very small crystal, smaller in fact than the size normally used. Darwin, however, pointed out that most ordinary macroscopic crystals behave like a mosaic of small blocks; thus the formula, which Darwin himself derived, is applicable in most cases. A similar formula was later deduced independently by Ewald.

The effects of primary and secondary extinction may be considerable. Primary extinction is caused by reflection of the incident x-ray beam from surface layers of crystals with large regions of perfect crystal structure, while secondary extinction is due to "screening" of the

lower blocks of the mosaic structure from the incident beam by outer blocks.

1. (8) THE PHASE PROBLEM AND THE HEAVY ATOM METHOD.

The electron density at any point in the crystal lattice can be evaluated by summing a Fourier series in which the coefficients are the structure factors. The amplitude of the structure factors can be obtained from their relationship with the integrated intensity; the phase on the other hand cannot be determined experimentally. The determination of the unknown phases is the central problem of x-ray structure analysis, and for a specific crystal, no general method of finding the phases exists.

One method of overcoming this problem is to prepare by chemical methods compounds which contain one or more atoms of greater atomic number than the other atoms (Robertson and Woodward, 1937; 1940). The positions of such atoms may be determined from the Patterson function and be used to calculate a phase for each reflection. As these atoms are heavier than the remainder, they make major contributions to each structure factor and an approximation to the true electron density function is obtained by summing a Fourier series with the observed structure amplitudes and the calculated phases. From the electron density

distribution, atomic positions of some, if not all, of the remaining atoms are found. The process of phase determination is repeated with the inclusion of the new atomic positions until a reliable set of phases is determined.

Clearly, the heavier the phase determining atom, the more accurately are the phases determined, but it is inadvisable that the scattering factor of the heavy atom should be too large compared with the scattering factors of the light atoms, otherwise the lighter atoms will be seen only with difficulty in a Fourier synthesis based on the heavy atom phases alone.

The phase-determining power of the heavy atom is a function of the ratio $(f_H^2/\sum f_L^2)$, where f_L and f_H refer to the scattering factors of light and heavy atoms, respectively. For the centrosymmetrical case the fraction of signs of the structure factors, F, determined by the heavy atom contributions, F_H, has been evaluated as a function of $f_H^2/\sum f_L^2$ by Sim (1957); when $f_H^2 = \sum f_L^2$ about 80% of the signs given by the heavy atom contributions are correct. Lipson and Cochran (1957) have suggested that when $f_H^2/\sum f_L^2 = 1$ the heavy-atom method should work most effectively.

In the non-centrosymmetrical case, there is a continuous distribution of errors, some large, some small

and the detailed distribution is given by $r = (f_H^2/\Sigma f_L^2)^{\frac{1}{2}}$. For the simplest case of one heavy atom and a number of light atoms in a triclinic cell, the manner in which the fraction $N(\xi)$ of structure factors with phase-angle errors between $\pm\xi$ varies as r varies can be calculated (Sim, 1957). When $r = 1$, 38% of the errors lie in the range $\pm 20^\circ$ while when $r = 2$, 67% of the errors lie in the same range; the error is defined as $\alpha - \alpha_H$ where α is the true phase angle of a structure factor, and α_H the phase angle calculated on the basis of the heavy atom.

The possibility of phase determination also occurs if two isomorphous crystals are available in which there is one replaceable heavy atom. An early example of this is the elucidation of the structure of phthalocyanine by investigation of the metal-free and nickel compounds (Robertson, 1935; 1936).

1. (9) THE PATTERSON FUNCTION

Suppose $(x + u, y + v, z + w)$, the distribution of scattering material about (xyz), is a function of u, v and w, and represents a distribution similar to $\rho(xyz)$ but displaced from (xyz) by the parameters u, v and w. The product $\rho(xyz)\ \rho(x + u, y + v, z + w)$ gives a representation of all the interatomic vectors in the unit cell, and will

have large values if u, v and w are the components of distance between two atoms in the structure.

P(uvw), a measure of the degree of coincidence of the two distributions, is given by

$$P(uvw) = V\int_{000}^{111}\int\int \rho(xyz)\,\rho(x+u, y+v, z+w)\,dxdydz.$$

The importance of this function becomes apparent when the Fourier expression for the electron density is substituted in the equation.

$$P(uvw) = \frac{1}{V}\int_{000}^{111}\int\int \sum_h\sum_k\sum_\ell\sum_{h'}\sum_{k'}\sum_{\ell'=-\infty}^{\infty} F(hk\ell)\exp.\left\{-2\pi i(hx+ky+\ell z)\right\}$$
$$\times F(h'k'\ell')\exp.\left\{-2\pi i(h'x+k'y+\ell' z)\right\}\exp.$$
$$\left\{-2\pi i(h'u+k'v+\ell' w)\right\}dxdydz.$$

The integral vanishes unless $h = h'$, $k = k'$, $\ell = \ell'$, when

$$P(uvw) = \frac{1}{V}\sum_h\sum_k\sum_{\ell=-\infty}^{\infty} F(hk\ell)\,F(\bar{h}\bar{k}\bar{\ell})\exp.\left\{2\pi i(hu+kv+\ell z)\right\}$$

The terms $F(hk\ell)$ and $F(\bar{h}\bar{k}\bar{\ell})$ are complex conjugates,

$$\therefore P(uvw) = \frac{1}{V}\sum_h\sum_k\sum_{\ell=-\infty}^{\infty} |F(hk\ell)|^2\exp.\left\{2\pi i(hu+kv+\ell z)\right\}$$

That the quantity P(uvw) is real for all values of u, v and w can be shown by collecting together the coefficients in pairs, $hk\ell$ and $\bar{h}\bar{k}\bar{\ell}$, viz.

$$P(uvw) = \frac{1}{2V}\sum\sum\sum_{-\infty}^{+\infty}|F(hk\ell)|^2\exp.\left\{-2\pi i(hu+kv+\ell w)\right\}$$
$$+ |F(\bar{h}\bar{k}\bar{\ell})|^2\exp.\left\{2\pi i(hu+kv+\ell w)\right\}$$
$$= \frac{1}{2V}\sum\sum\sum_{-\infty}^{+\infty}|F(hk\ell)|^2\left[\exp.\left\{-2\pi i(hu+kv+\ell w)\right\}\right.$$
$$\left. + \exp.\left\{2\pi i(hu+kv+\ell w)\right\}\right]$$

since $|F(\bar{h}\bar{k}\bar{l})| = |F(hkl)|$.

Thus $P(uvw) = \frac{1}{V}\sum_{-\infty}^{+\infty}\sum\sum|F(hkl)|^2\cos 2\pi(hu + kv + lw)$.

This series, deduced by Patterson (1934; 1935), incorporates the square of the structure amplitude in place of the structure factor as Fourier coefficient.

The distribution of P(uvw) has the same unit cell dimensions as the true unit cell, and has a large peak at the origin caused by the products of all the atoms with themselves. The height of the peak at the point (uvw) will be relatively large if u, v and w are components of distance between heavy atoms, and the positions of the heavy atoms in the unit cell can be determined, providing a starting point in the solution of the phase problem.

1. (10) REFINEMENT

After a structure has been solved, it is necessary to adjust the atomic parameters for each atom to give the best agreement between observed and calculated structure amplitudes. The process of improvement is known as refinement of a structure.

This may be done in the first place using Fourier syntheses from which new atomic positions are calculated, and employing these in further cycles of structure factor calculation.

An infinite number of terms ought to be summed in the Fourier series, but the use of x-rays of wavelength about 1.5 Å places a severe limitation on the amount of data which can be collected. The result of having a finite number of terms is that the peaks, although perfectly resolved, are surrounded by diffraction ripples which cause the observed peaks to be displaced from their true positions. This is known as the termination-of-series error.

The error may be rectified using the "back-shift" correction. Two Fourier series are computed, one using observed structure amplitudes (Fo) as coefficients, the other, calculated structure amplitudes (Fc). If there are no errors due to termination-of-series effects, the positions of the peaks in the Fc map will be identical with the sites from which the phases were calculated. In practice termination-of-series effects are present, and since the same number of terms are employed in both summations, the deviations of peaks in the Fc map from the sites used to calculate the phases give a measure of the termination-of-series errors in the Fo map. Hence the deviations of the peaks from these sites, Δx, Δy and Δz, represent the correction with changes of sign, to be applied to the positions of peaks in the Fo map.

The second method of refinement used extensively in this thesis is that based on the least-squares technique derived from the theory of errors.

If the parameters defining the structure are $u_1, u_2, \ldots\ldots, u_n$, then the calculated structure factor is some function of these parameters

$$Fc = f(u_1, u_2, \ldots\ldots u_n)$$

A similar expression holds for the observed structure amplitude

$$Fo = f(u_1 + \varepsilon_1, u_2 + \varepsilon_2, \ldots\ldots, u_n + \varepsilon_n)$$

where $\varepsilon_1, \varepsilon_2, \ldots\ldots, \varepsilon_n$, are the shifts required to give the true structural parameters.

If the initial structure is a good approximation then $\varepsilon_1, \varepsilon_2, \ldots\ldots, \varepsilon_n$ will all be small and the equation involving Fo can be expanded by Taylor's series.

$$Fo = f(u_1, u_2, \ldots\ldots, u_n) + \sum_{i=1}^{n} \frac{\partial f(u_1, u_2, \ldots\ldots, u_n)}{\partial u_i} \varepsilon_i$$

where the second and higher order differentials are neglected

i.e. $$Fo = Fc + \sum_{i=1}^{n} \frac{\partial Fc}{\partial u_i} \varepsilon_i$$

$$\therefore (Fo - Fc) = \sum_{i=1}^{n} \frac{\partial Fc}{\partial u_i} \varepsilon_i$$

An equation of this type may be derived for all the reflections, and the theory of errors predicts that the most acceptable set of $_i$ values is that which minimises the weighted sum of squares of the errors, (|Fo| - |Fc|),

$$\text{i.e.} \sum_h \sum_k \sum_l \omega(|Fo| - |Fc|)^2 = \sum_{hkl} \omega\Delta^2$$

where the weighting factor ω depends on the reliability of the observed structure amplitudes.

The criterion that $\sum \omega \Delta^2$ should be a minimum leads to a set of simultaneous equations, the normal equations

$$\sum_{hkl} \omega \frac{\partial Fc}{\partial u_i} = \sum_{hkl} \omega \left(\frac{\partial Fc}{\partial u_i}\right)^2 \epsilon_i + \sum_{hkl} \omega \frac{\partial Fc}{\partial u_i} \left(\sum_{j \neq i}^{n} \frac{\partial Fc}{\partial u_j} \epsilon_j \right)$$

If the parameters are all referred to orthogonal axes and there is sufficient data to resolve atoms clearly, quantities of the form

$$\sum_{hkl} \omega \frac{\partial Fc}{\partial u_i} \left(\sum_{j \neq i} \frac{\partial Fc}{\partial u_j} \epsilon_j \right)$$

are likely to be small compared with

$$\sum_{hkl} \omega \left(\frac{\partial Fc}{\partial u_i}\right) \epsilon_i$$

and may be neglected.

The solution of the equations now becomes

$$\epsilon_i = \frac{\sum_{hkl} \omega \Delta \frac{\partial Fc}{\partial u_i}}{\sum_{hkl} \omega \left(\frac{\partial Fc}{\partial u_i}\right)^2}$$

The least-squares programme available for the DEUCE computer refines three positional parameters per atom, six thermal parameters per atom, and an overall scale factor. The programme solves the normal equations using 3 x 3 and 6 x 6 block diagonal matrices for the positional and thermal parameters respectively of each atom in turn, and a 2 x 2 matrix for the scale factor (Rollett, 1961).

When only the diagonal elements of the least-squares matrix are employed, the standard deviations of the quantities u_i can be found from the totals $\sum\omega\left(\frac{\partial Fc}{\partial u_i}\right)^2$ viz.

$$(u_i)^2 = \frac{\sum\omega\Delta^2}{(N-S)\sum\omega\frac{\partial Fc}{\partial u_i}^2}$$

In the analyses described in this thesis, the approximate expression given above was employed to calculate standard deviations of atomic positions.

PART II.

THE STRUCTURE OF HUNTERBURNINE ;

X-RAY ANALYSIS OF HUNTERBURNINE

β - METHIODIDE

2. (1) INTRODUCTION

During the course of an examination of Hunteria Eburnea Pichon for substances of therapeutic value, several new alkaloids were isolated, some of which were tertiary (Bartlett and Taylor, 1960) and some quaternary bases (Bartlett, Sklar, Smith and Taylor).

Three of the quaternary bases have been shown by classical organic methods to be indole alkaloids of the yohimbine type. Also among the quaternary bases is a group of isomeric compounds, $C_{20}H_{27-29}O_2ClN_2$, all of which contain a 5-hydroxyindole chromophore, an isolated double bond and an aliphatic hydroxyl group which is readily acetylated; paucity of material precludes any detailed structural investigation by degradative studies.

The methiodide of one of these compounds, hunterburnine β-methiodide, $C_{20}H_{27}O_2IN_2$, was subjected to x-ray analysis. In addition to the structural features already cited, a nine membered ring adjacent to the five membered ring of the indole system was postulated, and the double bond was assumed to reside in a vinyl group.

Crystals in the shape of slender white needles wer supplied by the CIBA group in America.

2. (2) EXPERIMENTAL

Rotation, oscillation and Weissenberg photographs were taken using copper-K_α ($\lambda = 1.542$Å) radiation, and precession photographs were taken with molybdenum-K_α ($\lambda = 0.7107$Å) radiation. Unit cell dimensions were evaluated from precession photographs. The space group was assigned from systematic absences.

For the intensity measurements small crystals were employed completely bathed in a uniform x-ray beam, and no corrections for absorption were applied. Intensity data for the hk0,.....,hk7 reciprocal lattice nets were collected from equatorial and equi-inclination upper-layer Weissenberg photographs using the multiple film technique (Robertson, 1943), and estimated visually by comparison with a calibrated intensity scale. The film factor used to correlate the intensities on successive films of a series in non-equatorial layers was calculated from

$$R = 1.29 \exp. \ (0.942 \sec. \nu)$$

where ν is the angle which the incident beam makes with the normal to the film (Rossmann, 1956). The intensities were corrected for Lorentz, polarization, and rotation factors for upper layers, and values of $|F_o|$ calculated from the mosaic-crystal formula.

The various zones were placed on the same relative scale by comparison with common reflections on the okℓ precession series. The absolute scale was obtained at a later stage by correlation with the final calculated structure amplitudes $|F_c|$. In all, 1792 independent structure amplitudes were measured, of which 170 were smaller than the least observable value.

The crystal density was determined by a flotation technique in a mixture of chloro- and bromobenzene.

2. (3) CRYSTAL DATA.

$C_{20}H_{27}O_2IN_2$ $M = 454.3$

Orthorhombic

$a = 10.96 \text{ Å} \pm 0.02 \text{ Å}$

$b = 18.83 \text{ Å} \pm 0.03 \text{ Å}$

$c = 9.20 \text{ Å} \pm 0.03 \text{ Å}$

Volume of the unit cell = 1899 Å^3

For $Z = 4$, ρ(calculated) = 1.589 g./ml.

ρ(measured) = 1.602 g./ml.

F(000) = 920

Absorption coefficient for x-rays ($\lambda = 1.542$) = 136.5 cm.^{-1}

Systematic absences: (h00) = 2n + 1

(0k0) = 2n + 1

(00ℓ) = 2n + 1

Space group $P2_12_12_1 - D_2^4$.

2. (4) LOCATION OF THE HEAVY ATOM

For a crystal belonging to the orthothombic system, the expression for the Patterson function, P(UVW) is

$$P(UVW) = \frac{8}{Vc}\sum_{000}^{\infty\infty\infty}|F(hk\ell)|^2\cos 2\pi hU\cos 2\pi kV\cos 2\pi \ell W$$

which can be simply reduced to a two-dimensional expression for projections.

The two-dimensional Patterson projections P(UV), P(VW) were computed with 242 and 194 co-efficients respectively; the resulting maps are shown in Figs. 4 and 5 respectively. The space group of the Patterson function in the orthorhombic system is Pmmm and the mirror planes at U, V and W equal to $\frac{1}{2}$ obviate the computation of the whole unit cell.

In the projection down (100) the vectors expected between iodine atoms in the quadrant computed, assuming one iodine per assymetric unit, are at $(2y_I, \frac{1}{2})$, $(\frac{1}{2}, \frac{1}{2} - 2z_I)$ and $(\frac{1}{2} - 2y_I, 2z_I)$, the first two being double weight peaks, and the latter, single weight. (For a comprehensive scheme of vectors arising in the space group $P2_12_12_1$ see Chapter 3. (4)). These vectors are clearly distinguishable in Fig. 5, and are marked A, B and C respectively.

In the quadrant of the P(UV) projection computed, there should be peaks of double weight at $(\frac{1}{2}, \frac{1}{2} - 2y_I)$ on the line $P(\frac{1}{2}, V)$ and at $(2x_I, \frac{1}{2})$ on the line $P(U, \frac{1}{2})$, with a single weight peak at $(\frac{1}{2} - 2x_I, 2y_I)$ in a general

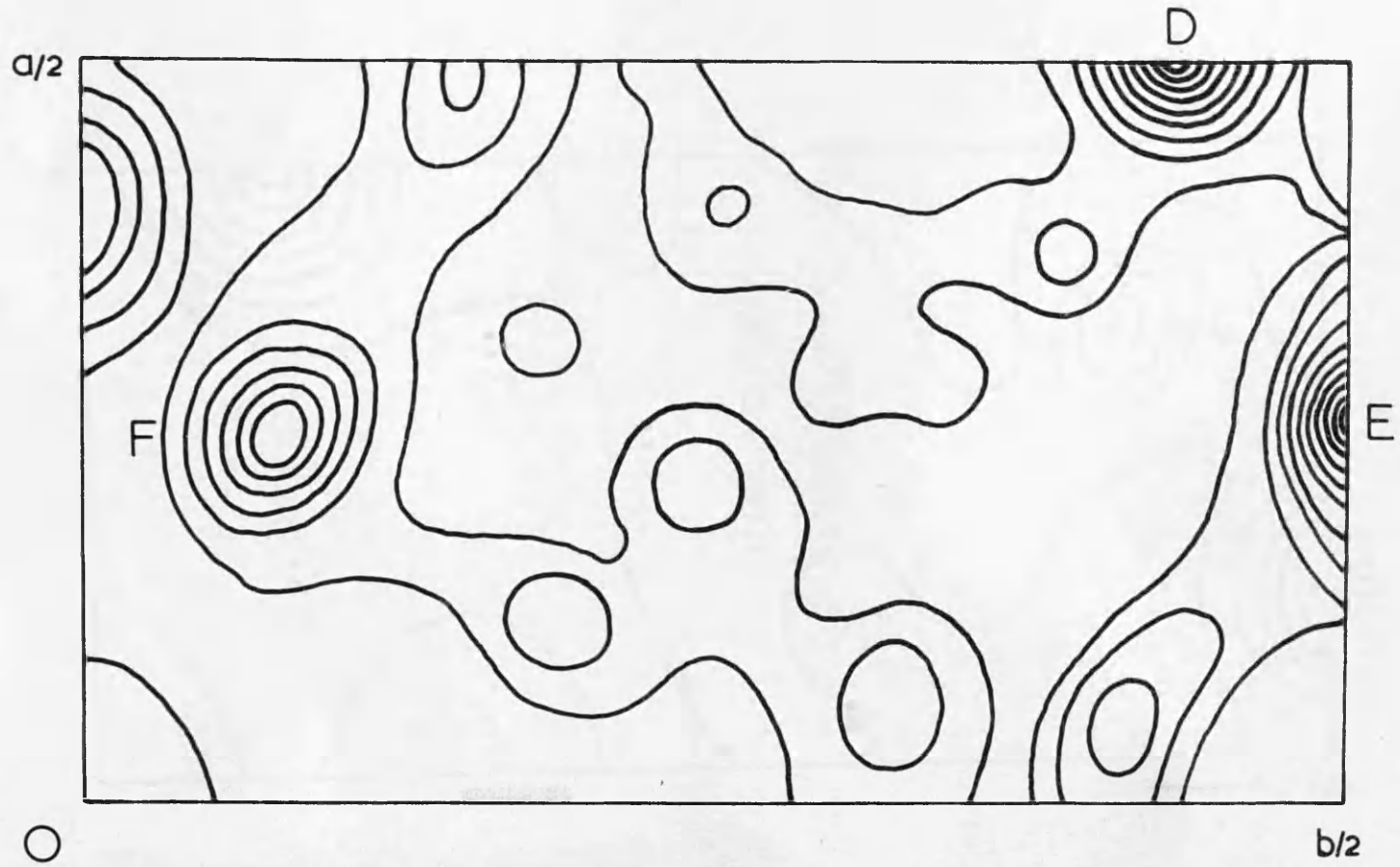


Fig.4. Hunterbrunine β-methiodide.
(00ℓ) Patterson projection. Contours are at equal and arbitrary intervals. I-I vectors discussed in the text are marked D, E and F.

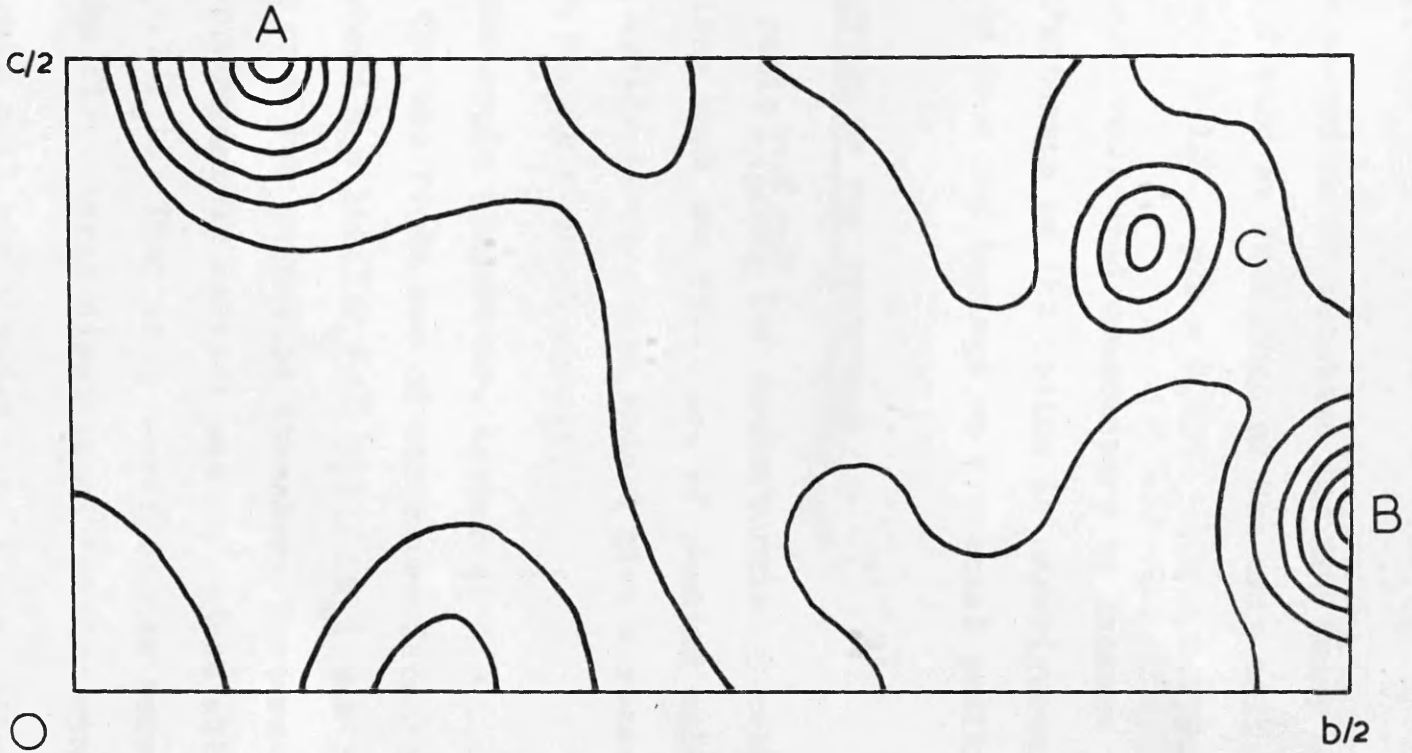


Fig.5. Hunterbrunine β-methiodide

(100) Patterson projection. Contours are at equal and arbitary. I-I vectors discussed in the text are marked A, B and C.

position. These vectors are marked D, E and F respectively in Fig. 4.

The co-ordinates obtained for the heavy atom in this way, expressed as fractions of the unit cell edges are

$$x/a = 0.127 \quad y/b = 0.037 \quad z/c = 0.179.$$

It was considered unnecessary to examine the three-dimensional Patterson as the iodine is unambiguously determined and does not impinge on a special position.

2. (5) SOLUTION OF THE STRUCTURE.

The ratio $\Sigma f_H^2/\Sigma f_L^2$ for hunterburnine β-methiodide is 2.97, indicating that the first set of phasing calculations based on the iodine atoms alone should give a reasonable approximation to the correct phases.

An isotropic temperature factor of $B_0 = 4.4$ Å^2 was assumed, and for the first set of structure factors, the residual R, where $R = 100(\Sigma|F_o| - \Sigma|F_c|)/\Sigma|F_o|$, was 32%. The criterion used for selecting structure factors whose phases were approximately correct was to reject all those for which $|F_o| > 2|F_c|$. Thus 1632 coefficients were employed in the first three-dimensional Fourier synthesis.

The unit cell was divided into a three-dimensional grid with intervals of 1/30 along a and c, and 1/60 along b, corresponding to intervals of 0.37 Å, 0.31 Å and 0.31 Å along a, b and c respectively. The electron density was

calculated at each grid point, contours mapped out on glass for consecutive sections of the grid, and the sections stacked up parallel to (001), giving a three-dimensional effect with atoms appearing in the unit cell as balls of high electron density.

A large number of positive electron-density concentrations was present in the first Fourier map, but none could be ascribed to atoms with any degree of certainty. The maxima of the fourteen most prominent were calculated by the method due to Booth (1946) and included as carbon atoms in a second set of phasing calculations. The discrepancy, R, dropped to 29%, and the subsequent three-dimensional Fourier synthesis furnished the complete structure with the exception of C(19) and C(21) which were poorly resolved, and C(14) for which alternative sites of equal peak height, affecting the stereochemistry of ring D, were present. These three excepted, all the light atoms were included, with each atom assigned its correct chemical type, in a third phasing calculation, when R fell to 27.4%. C(21) was clearly resolved in the third F_o synthesis and included in a fourth structure factor calculation.

The discrepancy resulting was 24.3%, and a fourth three-dimensional electron density distribution based on the revised phases was calculated. The position of C(14)

was clearly established, but C(19) was not yet revealed, and it was noted that C(18) had a very low peak height. Alternative sites for C(18), and C(19) attached to C(20) were evident, and on calculating a further set of structure factors with the exclusion of C(18) and C(19), the value of R fell to 20.6%. Examination of the electron-density distribution of the fifth three-dimensional Fourier synthesis allowed confident assignation of C(18) and C(19), the latter attached to C(20).

This completed the determination of the structure, and its course is shown in Table 1.

2. (6) REFINEMENT

The location and contribution of hydrogen atoms were not considered at any stage of the refinement. The course of the refinement is recorded in Table 2.

Two cycles of structure factor and Fourier calculation were carried out and back-shift corrections for errors due to termination of series were applied to the atomic coordinates. Isotropic temperature factors were assigned individually to each atom from a comparison of peak heights in the F_o and F_c syntheses. The value of R fell to 18.2%.

TABLE I.

Hunterburnine β-methiodide

Course of the Structure Determination

Operation	Atoms included	R(%)
2D Patterson synthesis		-
1st 3D Fourier synthesis	I	32.2
2nd " " "	I+14(C)	29.2
3rd " " "	I+17(C)+2(N)+2(O)	27.4
4th " " "	I+18(C)+2(N)+2(O)	24.3
5th " " "	I+18(C)+2(N)+2(O)	20.6

TABLE 2.

Hunterburnine β-methiodide

Course of Refinement

Operation	Atoms included	R(%)	$\Sigma\omega\Delta^2$
6th 3D Fourier synthesis (F_o and F_c)	I+20(C)+2(N)+2(O)	18.4	-
7th 3D Fourier synthesis (F_o and F_c)	I+20(C)+2(N)+2(O)	18.2	-
1st Least-squares cycle	I+20(C)+2(N)+2(O)	18.1	1457
2nd " " "	I+20(C)+2(N)+2(O)	17.2	1413
3rd " " "	I+20(C)+2(N)+2(O)	16.4	581
4th " " "	I+20(C)+2(N)+2(O)	15.8	486
5th " " "	I+20(C)+2(N)+2(O)	15.5	459
6th " " "	I+20(C)+2(N)+2(O)	15.4	453
8th 3D Fourier synthesis	I+20(C)+2(N)+2(O)	15.4	-

The next stage of refinement consisted of adjustment of the atomic coordinates and anisotropic temperature factors by the least squares method. Only the block diagonal elements of the matrix of normal equations were used. The weighting system used was a simple function of F_o (Rollett, 1961).

$\sqrt{\omega} = |F_o| / F^*$ if $|F_o| \leq F^*$; $\sqrt{\omega} = F^* / |F_o|$ if $F^* < |F_o|$.

where F* is a constant: in this case F* = 15.

Half-scale scattering factors were used to ensure that the equations involving the iodine atom would solve. In the second cycle, however, the iodine equations failed to solve and subsequently quarter scale scattering factors were employed. Half-shift corrections were used throughout the refinement.

The reduction in $\Sigma\omega\Delta^2$ from cycle V to cycle VI was small, and refinement was terminated at that stage. The final value of the discrepancy, R, excluding unobserved terms, was 15.4%.

For the structure factor calculations throughout, theoretical atomic scattering factors were used: those of Berghius et al. (1935) for carbon, nitrogen and oxygen, and the Thomas-Fermi values (1935) for iodine were chosen.

2. (7) RESULTS

Final calculated and observed structure amplitudes and phase angles are recorded in Table 3. The final positional parameters are listed in Table 4. The final anisotropic temperature factor parameters given by the least-squares refinement are shown in Table 5; they are the values of b_{ij} in the equation

$$\exp.(-B\sin^2\theta/\lambda^2) = 2^{-(b_{11}h^2+b_{12}hk+b_{13}h\ell+b_{22}k^2+b_{23}k\ell+b_{33}\ell^2)}$$

Intramolecular bonded distances, intramolecular non-bonded distances $<4\text{Å}$, intermolecular distances $<4\text{Å}$ and inter-bond angles are compiled in Tables 6, 7, 8 and 9 respectively.

The standard deviations of the final atomic co-ordinates were derived from the least-squares residuals by application of the equation

$$\sigma^2(x_i) = \sum_j \omega_j(\Delta F_j)/\left\{(N-S)\sum_j \omega_j(\partial F_j/\partial x_i)^2\right\}$$

The results appear in Table 10.

The standard deviation, $\sigma(A-B)$, of a bond between atoms (A) and (B) is given by the formula

$$\sigma(A-B)^2 = \sigma_A^2 + \sigma_B^2$$

where $\sigma(A)$ and $\sigma(B)$ are the standard deviations in atomic co-ordinates of the atoms A and B. The standard deviation, $\sigma(\beta)$, for an angle (β) formed at atom (B) between two bonds AB and BC is evaluated (Cruickshank and Robertson, 1953) from

$$\sigma^2(\beta) = \frac{\sigma^2(A)}{(AB)^2} + \sigma^2(B)\left[\frac{1}{AB^2} - \frac{2\cos\beta}{AB.BC} + \frac{1}{(BC)^2}\right] + \frac{\sigma^2(C)}{(BC)^2}$$

Table 3. Hunterburnine β-methiodide.

Final measured and calculated values of the structure factors. Unobserved reflections have been omitted.

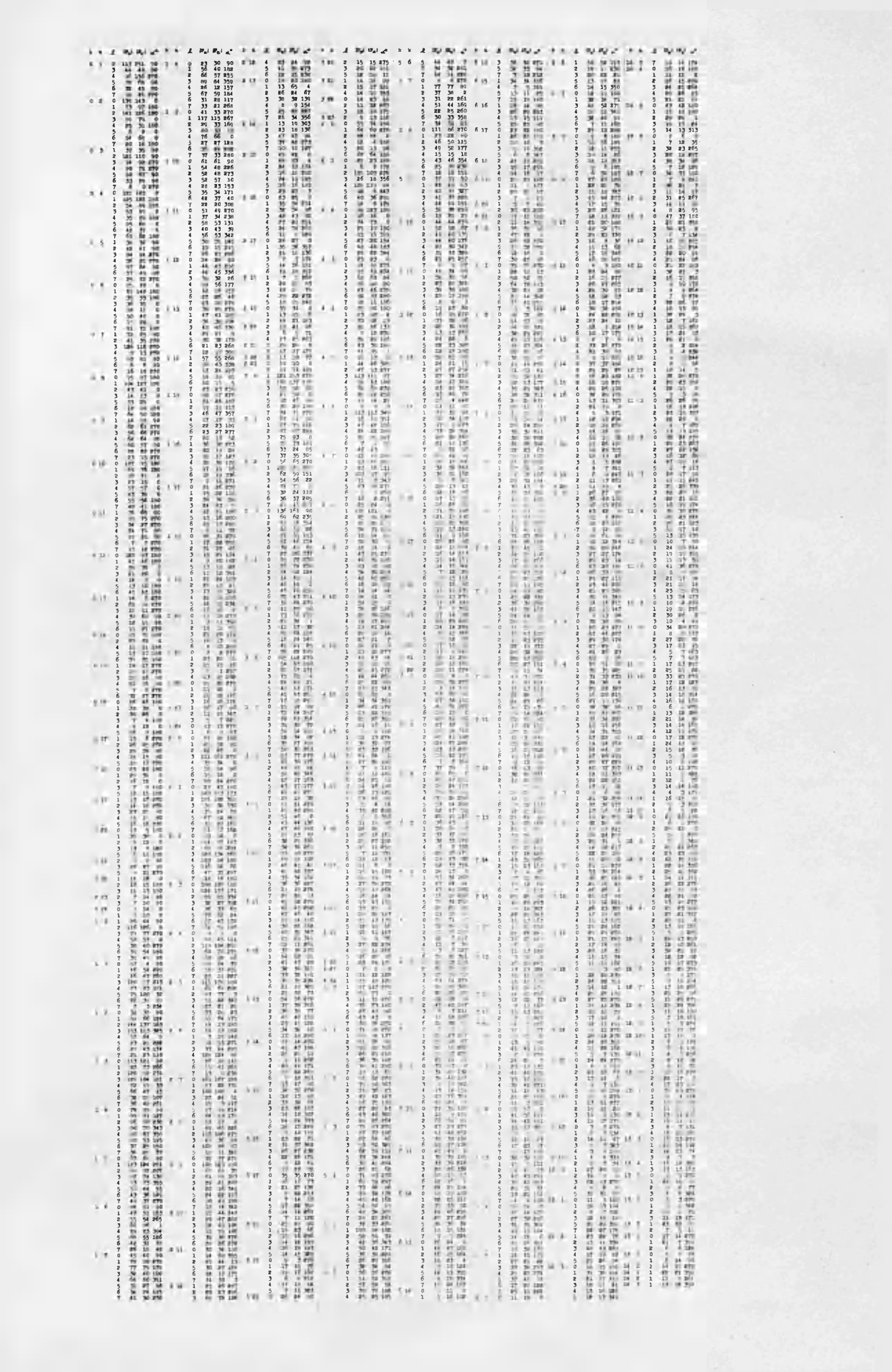


TABLE 4.

Hunterburnine β-methiodide

Final Atomic Coordinates

Atom	x/a	y/b	z/c
N(1)	0.8259	-0.0522	0.1763
C(2)	0.7180	-0.0311	0.1392
C(3)	0.6798	0.0541	0.1397
N(4)	0.5446	0.0560	0.1726
C(5)	0.4756	0.0107	0.0597
C(6)	0.4951	-0.0678	0.0867
C(7)	0.6347	-0.0776	0.1195
C(8)	0.7008	-0.1499	0.1147
C(9)	0.6679	-0.2153	0.1034
C(10)	0.7605	-0.2675	0.1277
C(11)	0.8765	-0.2466	0.1508
C(12)	0.9158	-0.1793	0.1878
C(13)	0.8274	-0.1312	0.1623
C(14)	0.7663	0.1015	0.2492
C(15)	0.7237	0.1764	0.2116
C(16)	0.5833	0.1809	0.2387
C(17)	0.5051	0.1360	0.1667
C(18)	0.7490	0.3298	0.1722
C(19)	0.7482	0.2923	0.0660
C(20)	0.7679	0.2065	0.0582
C(21)	0.9061	0.1945	0.0238
C(22)	0.5176	0.0317	0.3401
O(23)	0.9134	0.1184	-0.0264
O(24)	0.7213	-0.3397	0.1020
I	0.1278	0.0357	0.1841

TABLE 5.

Hinterburnine β-methiodide.

Final Anisotropic Thermal Parameters ($b_{ij} \times 10^{-5}$)

Atom	b_{11}	b_{22}	b_{33}	b_{12}	b_{23}	b_{13}
N(1)	876	349	3095	-62	-12	-2268
C(2)	856	407	2129	424	1137	1605
C(3)	518	296	2220	-404	221	-817
N(4)	665	499	1989	731	633	54
C(5)	1099	570	2055	715	-1099	-1203
C(6)	1389	508	2630	-135	1337	982
C(7)	945	399	1517	-179	701	1325
C(8)	1232	558	2091	-73	-514	1303
C(9)	1155	144	3515	-325	-1049	838
C(10)	1795	492	666	-538	-560	683
C(11)	1806	430	359	870	1185	-475
C(12)	1406	423	888	441	-404	165
C(13)	672	714	826	-112	-653	-155

Atom	b_{11}	b_{22}	b_{33}	b_{12}	b_{23}	b_{13}
C(14)	1759	317	1143	-132	936	-1716
C(15)	2596	287	824	436	1045	-40
C(16)	2847	361	1133	877	1261	-1284
C(17)	1948	322	1825	529	368	550
C(18)	1995	628	793	-238	-1375	448
C(19)	1800	607	4071	137	686	-1778
C(20)	788	151	2570	113	179	941
C(21)	2401	337	1832	452	-378	1693
C(22)	1835	748	222	42	-482	1217
.O(23)	2607	892	1760	354	1397	2067
.O(24)	1649	296	4086	-289	-81	-616
I	1119	418	1887	-25	-137	-54
($B = 5A^{o2}$)	1501	508	2130	-	-	-

TABLE 6.

Hunterburnine β-methiodide

Intramolecular Bonded Distances (Å)

N(1) - C(2)	1.29	C(9) - C(10)	1.43
N(1) - C(13)	1.49	C(10) - C(11)	1.35
C(2) - C(3)	1.66	C(10) - O(24)	1.45
C(2) - C(7)	1.28	C(11) - C(12)	1.38
C(3) - N(4)	1.51	C(12) - C(13)	1.35
C(3) - C(14)	1.65	C(14) - C(15)	1.53
N(4) - C(5)	1.54	C(15) - C(16)	1.56
N(4) - C(17)	1.57	C(15) - C(20)	1.60
N(4) - C(22)	1.64	C(16) - C(17)	1.37
C(5) - C(6)	1.51	C(18) - C(19)	1.21
C(6) - C(7)	1.57	C(19) - C(20)	1.63
C(7) - C(8)	1.54	C(20) - C(21)	1.56
C(8) - C(9)	1.29	C(21) - O(23)	1.51
C(8) - C(13)	1.50		

TABLE 7.

Hunterburnine β-methiodide

Intramolecular Non-bonded Distances <4Å.

N(1) - N(4)	3.70	C(6) - C(13)	3.90
N(1) - C(6)	3.73	C(6) - C(17)	3.90
N(1) - C(9)	3.59	C(6) - C(22)	3.00
N(1) - C(11)	3.71	C(7) - C(10)	3.83
N(1) - C(14)	3.04	C(7) - C(12)	3.68
N(1) - O(23)	3.84	C(7) - C(14)	3.86
C(2) - C(5)	2.87	C(7) - C(22)	3.16
C(2) - C(9)	3.53	C(8) - C(11)	2.67
C(2) - C(12)	3.56	C(8) - O(24)	3.58
C(2) - C(15)	3.97	C(9) - C(12)	2.91
C(2) - C(17)	3.92	C(10) - C(13)	2.69
C(2) - C(22)	3.11	C(12) - O(24)	3.78
C(2) - O(23)	3.85	C(14) - C(17)	3.05
C(3) - C(6)	3.10	C(14) - C(19)	3.97
C(3) - C(8)	3.86	C(14) - C(21)	3.12
C(3) - C(13)	3.85	C(14) - C(22)	3.14
C(3) - C(16)	2.76	C(14) - O(23)	3.02
C(3) - C(20)	3.12	C(15) - C(18)	2.92
C(3) - C(21)	3.78	C(15) - C(22)	3.73
C(3) - O(23)	3.22	C(15) - O(23)	3.21
N(4) - C(7)	2.75	C(16) - C(18)	3.40
N(4) - C(15)	3.02	C(16) - C(19)	3.19
N(4) - C(20)	3.89	C(16) - C(22)	3.05
C(5) - C(8)	3.94	C(17) - C(20)	3.31
C(5) - C(16)	3.79	C(18) - C(21)	3.37
C(6) - C(9)	3.37	C(19) - O(23)	3.84

TABLE 8.

Hunterburnine β-methiodide

Intermolecular Distances <4Å.

C(22) ...	$O(23)_{iii}$	3.17	C(6) ...	$O(23)_{iii}$	3.82
C(17) ...	$O(24)_{vi}$	3.34	C(14) ..	$C(2)_{iii}$	3.83
C(7) ...	$O(23)_{iii}$	3.39	C(15) ..	$C(8)_{iii}$	3.84
O(23) ...	I_i	3.42	C(21) ..	$C(19)_{iv}$	3.85
I ...	$O(24)_{vi}$	3.48	C(15) ..	$C(9)_{iii}$	3.87
C(14) ...	$C(8)_{iii}$	3.50	O(24) ..	$C(6)_{v}$	3.88
C(22) ...	$N(1)_{iii}$	3.56	C(21) ..	$C(16)_{iv}$	3.89
C(8) ...	$O(23)_{iii}$	3.58	C(22) ..	$C(13)_{iii}$	3.90
C(14) ...	$C(7)_{iii}$	3.61	C(13) ..	$O(23)_{iii}$	3.90
C(22) ...	$O(24)_{vi}$	3.61	C(17) ..	$C(10)_{vi}$	3.95
C(16) ...	$O(24)_{vi}$	3.67	C(14) ..	$C(9)_{iii}$	3.97
C(16) ...	$C(9)_{vi}$	3.68	C(9) ...	$O(23)_{iii}$	3.97
N(1) ...	I_i	3.70	C(9) ...	$C(21)_{iii}$	3.97
I ...	$C(5)_{ii}$	3.74	C(18) ..	$O(24)_{iii}$	3.97
C(19) ...	$C(17)_{iv}$	3.76	C(18) ..	$C(6)_{vi}$	3.98
C(22) ...	I_{ii}	3.77	C(14) ..	$C(13)_{iii}$	3.98
C(21) ...	$C(17)_{iv}$	3.77	I ..	$C(6)_{ii}$	3.99
C(2) ...	$O(23)_{iii}$	3.78	N(1) ...	$O(23)_{iii}$	3.99

The subscripts refer to the following positions:

i	$x + 1,$	$y,$	z	iv	$x + \frac{1}{2},$	$-y + \frac{1}{2},$	$-z$
ii	$-x + \frac{1}{2},$	$-y,$	$z + \frac{1}{2}$	v	$x + \frac{1}{2},$	$-y - \frac{1}{2},$	$-z$
iii	$-x + 1\frac{1}{2},$	$-y,$	$z + \frac{1}{2}$	vi	$-x + 1,$	$y + \frac{1}{2},$	$-z + \frac{1}{2}$

TABLE 9.

Hunterburnine β- methiodide.

Interbond Angles.

C(2) N(1) C(13)	107°	C(8) C(9) C(10)	117°
N(1) C(2) C(3)	122	C(9) C(10) C(11)	120
N(1) C(2) C(7)	119	C(9) C(10) O(24)	114
C(3) C(2) C(7)	119	C(11) C(10) O(24)	126
C(2) C(3) N(4)	106	C(10) C(11) C(12)	127
C(2) C(3) C(14)	115	C(11) C(12) C(13)	110
N(4) C(3) C(14)	112	N(1) C(13) C(8)	104
C(3) N(4) C(5)	109	N(1) C(13) C(12)	131
C(3) N(4) C(17)	106	C(8) C(13) C(12)	124
C(3) N(4) C(22)	111	C(3) C(14) C(15)	101
C(5) N(4) C(17)	110	C(14) C(15) C(16)	108
C(5) N(4) C(22)	113	C(14) C(15) C(20)	116
C(17) N(4) C(22)	107	C(16) C(15) C(20)	115
N(4) C(5) C(6)	111	C(15) C(16) C(17)	119
C(5) C(6) C(7)	107	N(4) C(17) C(16)	112
C(2) C(7) C(6)	130	C(18) C(19) C(20)	128
C(2) C(7) C(8)	106	C(15) C(20) C(19)	106
C(6) C(7) C(8)	124	C(15) C(20) C(21)	115
C(7) C(8) C(9)	135	C(19) C(20) C(21)	106
C(7) C(8) C(13)	103	C(20) C(21) O(23)	105
C(9) C(8) C(13)	121		

TABLE 10.

Hunterburnine β-methiodide

Standard Deviations of the Final Atomic Coordinates (Å)

Atom	σ(x)	σ(y)	σ(z)
N(1)	0.031	0.034	0.030
C(2)	0.029	0.025	0.034
C(3)	0.024	0.026	0.037
N(4)	0.023	0.025	0.034
C(5)	0.037	0.039	0.042
C(6)	0.032	0.028	0.041
C(7)	0.048	0.045	0.057
C(8)	0.043	0.038	0.037
C(9)	0.034	0.032	0.044
C(10)	0.038	0.042	0.042
C(11)	0.037	0.032	0.034
C(12)	0.041	0.043	0.048
C(13)	0.041	0.034	0.045
C(14)	0.029	0.036	0.033
C(15)	0.038	0.029	0.037
C(16)	0.044	0.031	0.034
C(17)	0.033	0.033	0.041
C(18)	0.030	0.039	0.036
C(19)	0.046	0.037	0.038
C(20)	0.038	0.046	0.033
C(21)	0.040	0.034	0.034
C(22)	0.045	0.033	0.037
O(23)	0.044	0.036	0.041
O(24)	0.042	0.038	0.040
I	0.009	0.002	0.003

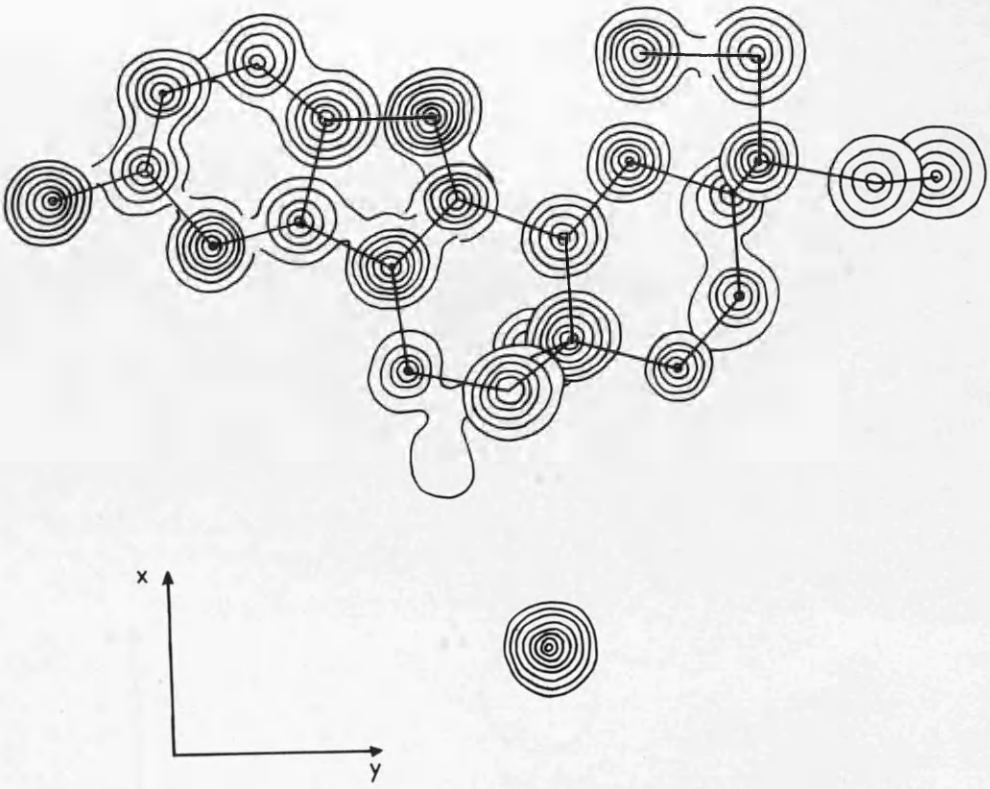


Fig. 6. Hunterburnine 6-methiodide.
Superimposed contoured sections of the final 3D Fourier synthesis covering the region of one molecule. The sections are drawn parallel to (001). Contours are drawn at intervals of 1 electron/Å^3 with the exception of the iodide ion for which the intervals are at 7 electrons/Å^3.

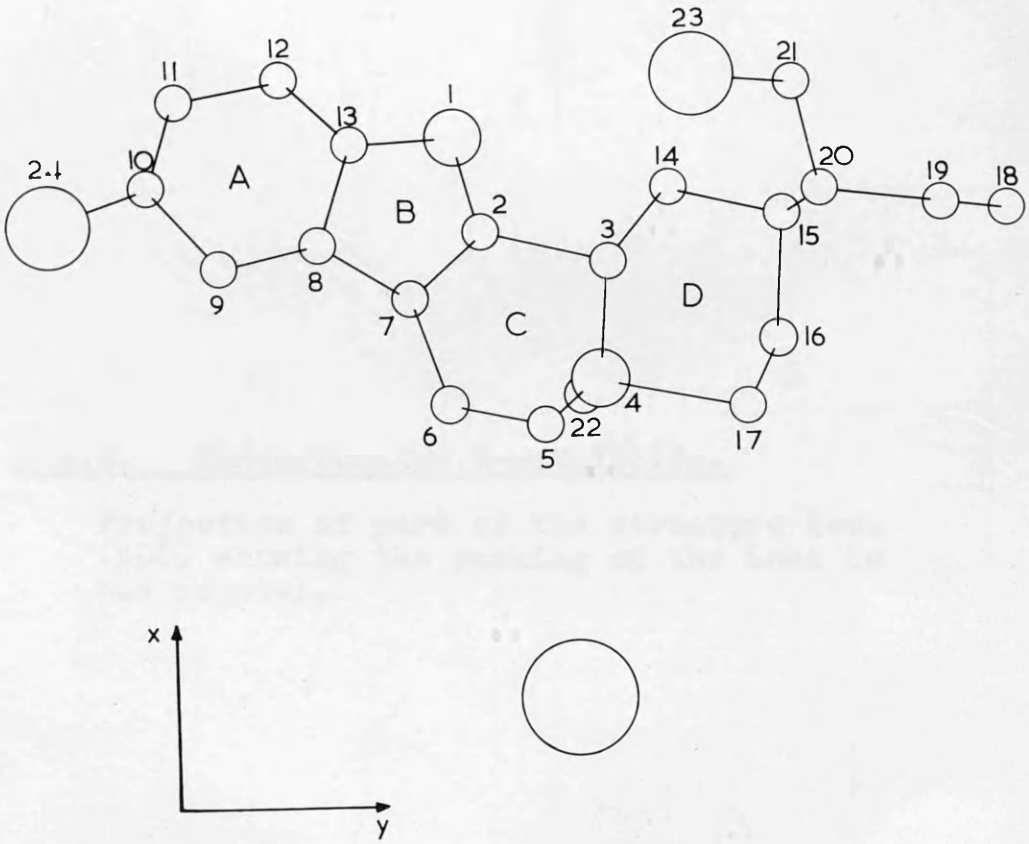
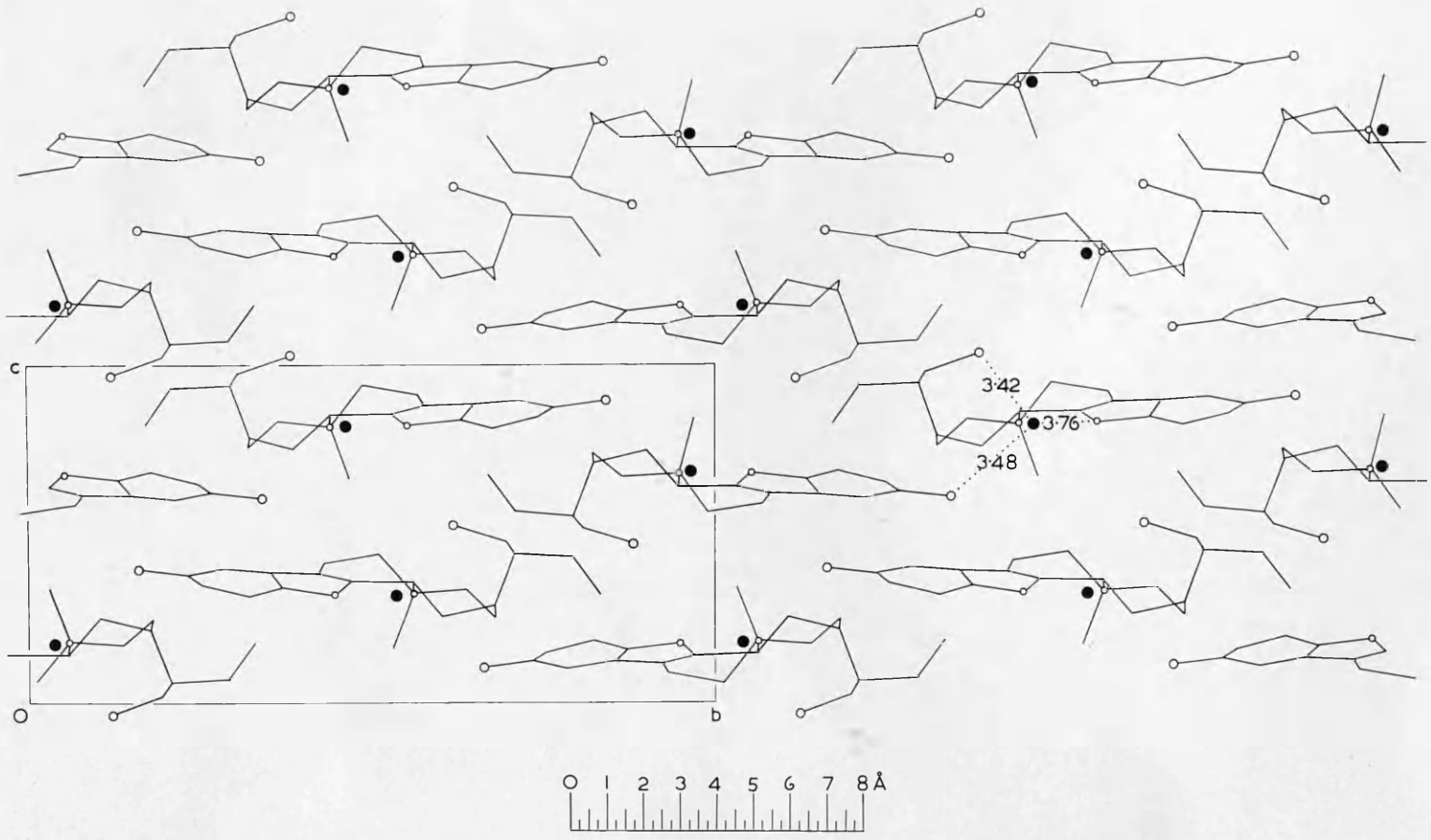


Fig. 7. Hunterbrunine S-methiodide.
Atomic arrangement corresponding to Fig. 6.

Fig.8. Hunterburnine β-methiodide.

Projection of part of the structure down (100) showing the packing of the ions in the crystal.

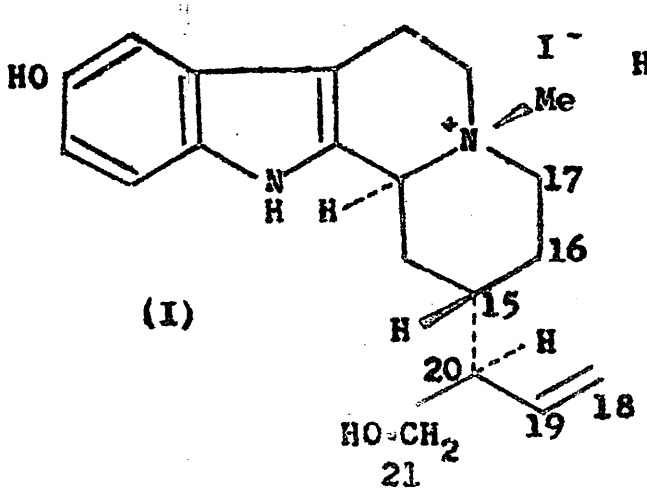

c
O
b
3·42
3·76
3·48
0 1 2 3 4 5 6 7 8 Å

In hunterburnine β-methiodide, the average estimated standard deviation of a distance between two light atoms (carbon, nitrogen or oxygen) is about 0.05 Å, and that of a C-I bond about 0.03 Å. The average standard deviation of a bond angle is about 3°.

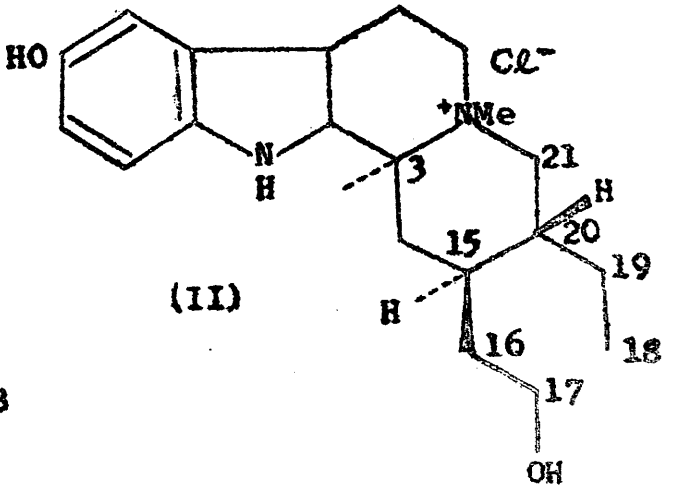
On the basis of the final phase constants, an eighth and final three-dimensional Fourier synthesis was evaluated, and the electron-density distribution over one molecule is delineated by means of superimposed contour sections drawn parallel to (001) in Fig. 6, and the corresponding atomic arrangement is drawn in Fig. 7. Fig. 8 shows the crystal structure of hunterburnine β-methiodide as viewed in projection down the (100) axis.

2. (8) DISCUSSION

The final results of the analysis establish the constitution and stereochemistry (apart from absolute stereochemistry) of hunterburnine β-methiodide to be as in (I)



(I)



(II)

The assignment of the double bond between C(18) and C(19) was based on examination of bond lengths and planarity of the C(18), C(19) and C(20) system, and was confirmed by nuclear magnetic resonance studies by the CIBA group. The methyl group on N(4) is β-orientated and the adjacent six-membered rings are transfused. The six-membered ring C(3), N(4), C(14), C(15), C(20) and C(21) adopts the chair conformation.

Hunterburnine β-methiodide is the first recognised representative of a new class of indole alkaloid. The biogenetic relationship to other indole alkaloids becomes clear when it is realised that the skeleton of hunterburnine β-methiodide can be derived from that of dihydrocorynantheol methochloride (II) - also isolated from Hunteria Eburnea - by an appropriate scission and recyclisation as indicated by the numbering in the formula. The absolute stereochemistry indicated in (I) is based on the assumption that the rule of uniform absolute stereochemistry of the C(15) equivalent of yohimbine - C(15) in (I) and (II) - is valid in this case. (Wenkert and Bringi, 1959). ψ-akuammicine is the only known exception to the rule (Edwards and Smith, 1960).

The crystal-structure analysis of hunterburnine α-methiodide which is the N(b) -epimer of (I) has recently been carried out (Scott, Sim and Robertson, 1962). Though

synthetic N(b)-epimeric yohimbine methiodides are known (Witkop and Goodwin, 1953), it is noteworthy that hunterburnine α-methiodide and hunterburnine β-methiodide constitute the first recognised pair of naturally occurring N(b)-epimeric quaternary alkaloids. There is an interesting pharmacological difference between the compounds, the α-methiodide inducing a marked lowering of blood pressure in the anaesthetized dog. (Taylor, 1962).

The molecular dimensions calculated from the final atomic coordinates (see Table 4) are not very precise. The presence of the iodine atoms, and the consequent absorption, is probably in large measure responsible for this.

The average lengths of the aromatic carbon-carbon bonds and carbon-carbon single bonds are 1.38 Å and 1.56 Å in reasonable agreement with the accepted values of 1.397 Å and 1.545 Å respectively (Sutton et al., 1958). The C(2)-C(7) double bond length, 1.28 Å, is not significantly shorter than the value of 1.334 Å attributed to ethylene (Sutton et al., 1958). The C(18)-C(19) double bond length of 1.21 Å appears to be significantly short, but examination of the bond lengths in the vinyl group shows that the C(19)-C(20) single bond is rather long, 1.63 Å, while the C(18)-C(19) double bond is short and it is likely that C(19) has been misplaced by the least-squares procedure.

The carbon-nitrogen bonds fall into two categories: $C(sp^2)$-N and $C(sp^3)$-N^+. The average $C(sp^2)$-N bond length of 1.39 Å agrees well with values reported for such bonds of 1.37 Å in p-nitroaniline (Trueblood, Goldish and Donohue, 1961), 1.395 Å in ibogaine hydrobromide (Arai, Coppola and Jeffrey, 1960) and 1.40 Å in calycanthine dihydrobromide dihydrate (Hamor and Robertson, 1962). The average $C(sp^3)$-N^+ bond length is 1.57 Å. The results of a number of x-ray measurements of such bonds in alkaloids indicate that long $C(sp^3)$-N^+ bonds are a common feature. Thus the average value in ibogaine hydrobromide is 1.53 Å, in (+)-demethanolaconinone hydriodide trihydrate, 1.54 Å (Przybylska, 1961), in echitamine bromide methanol solvate, 1.55 Å (Hamilton, Hamor, Robertson and Sim, 1962) and in codeine hydrobromide dihydrate, 1.53 Å (Lindsay and Barnes, 1955).

The means of the valency angles of the benzene and five-membered rings are 120° and 108° respectively, the values anticipated for planar rings. The average valency angle in ring C(see Fig. 7) is 114°, larger than tetrahedral due to the presence of the C(2)-C(7) double bond. The average value for the corresponding ring in macusine-A is 114° (McPhail, Robertson and Sim, 1963). In ring D, the average valency angle is 110°.

The equation of the mean plane through the indole system, calculated by the method of Schomaker et al. (1959)

is $0.241X + 0.071Y - 0.968Z - 5.643 = 0$, where X, Y and Z are coordinates expressed in Angstrom units referred to orthogonal axes a, b and c. The deviations from the plane are listed in Table II. Application of the χ^2 test indicates that they are possibly significant. No chemical or steric considerations provide any reason for this.

In the crystal, the positively charged hunterburnine molecules and iodide ions form a three-dimensional network held together by normal ionic forces, Van der Waals forces, and a system of hydrogen bonds involving the two oxygen atoms O(23) and O(24), the indole nitrogen atom N(1) and the iodide ion.

In the case of the oxygen atoms, the angles C(2) $O(23)I^1$ and $C(10)^2O(24)^2I$, where superscripts 1 and 2 refer to positions $x + 1, y, z$ and $\bar{x} + 1, \frac{1}{2} + y, \frac{1}{2} - z$ respectively, are 107^o and 114^o, close to the tetrahedral value. The OH ....I distances (3.42 Å and 3.48 Å) are similar to the hydrogen bonded distances of 3.57 Å in muscarine iodide (Jellinek, 1957), 3.43 Å in macusine-A iodide (McPhail, Robertson and Sim, 1963) and 3.52 - 3.62 Å in (+)-des-(oxymethylene)-lycoctonine hydriodide monohydrate (Przybylska, 1961).

It is probable that the hydrogen atom on the indole nitrogen, N(1), also takes part in hydrogen bonding. The

TABLE 11.

Hunterburnine β-methiodide.

Displacements (Å) of the Atoms of Hunterburnine β-methiodide. e System from the Mean Plane through N(1), C(2), C(7), C(8), C(9), C(10), C(11), C(12), C(13) and O(24).

N(1)	-0.023	C(9)	-0.008
C(2)	0.050	C(10)	-0.049
C(3)	0.058	C(11)	0.078
C(6)	-0.120	C(12)	-0.057
C(7)	-0.056	C(13)	0.001
C(8)	0.065	O(24)	-0.020

$\Sigma w\Delta^2 = 2.24 \times 10^{-2}$ $\chi^2 = 16.54$

$\sigma^2 = 13.5 \times 10^{-4}$ $P = 1\%$

angles C(2) N(1) I^1 and C(13) N(1) I^1 are 133° and 116° respectively while the NH ...I distance of 3.70 Å is close to that of 3.84 Å in (+)-des-(oxymethylene)-lycoctonine hydriodide monohydrate (Przybylska, 1961) and 3.5 Å in 2': 3'-isopropylidene, 3: 5' cycloadenosine iodide (Zussman, 1953).

The closest contacts between an iodide ion and carbon atoms, 3.74 Å, 3.77 Å, and 3.99 Å are similar to the minimum C....I distances of 3.93 Å and 3.95 Å in the crystal structure of macusine-A iodide (McPhail, Robertson and Sim, 1963), 3.81 Å in (+)-des-(oxymethylene)-lycoctonine hydriodide monohydrate (Przybylska, 1961) and 3.96 Å in isocryptopleurine methiodide (Fridrichsons and Mathieson, 1955).

Of the carbon-carbon intermolecular distances, sixteen are below 4 Å, ranging from 3.50 Å to 3.98 Å. There are three short van der Waals contacts between carbon and oxygen of 3.17 Å, 3.34 Å and 3.39 Å (Table 7) and this appears to be a normal occurrence for C....O contacts in alkaloids, as is evidenced by values of 3.18 Å in calycanthine dibromide dihydrate (Hamor and Robertson, 1962), 3.2 Å in (+)-demethanolaconinone (Przybylska, 1961) and 3.2 Å in (-)-aspisdospermine N(b)-methiodide (Mills and Nyburg, 1960). All other C....O approaches are greater than 3.6 Å.

PART III.

THE STRUCTURE OF ISOPHOTOSANTONIC LACTONE ; X - RAY ANALYSIS OF BROMODIHYDROISOPHOTO - α - SANTONIC LACTONE ACETATE

3 (1) INTRODUCTION

Homoannular cyclohexadienones are in general subject to rapid photochemical change, and the action of light on santonin (see Chapter 4. (1)) has been the subject of considerable study for some time. One of the irradiation products, isophoto-α-santonic lactone, was first isolated in Italy (Villavecchia, 1885) but its constitution was only recently established (Barton, de Mayo and Shafiq, 1957). Much of the stereochemistry, however, has not yet been elucidated.

The rearrangement has been found to be a general one in sesquiterpenoids, and an example has been encountered in the steroid series (Barton and Taylor, 1958).

There are five asymmetric centres in isophoto-α-santonic lactone: C(1), C(6), C(7), C(10) and C(11). None of the bonds attached to C(6), C(7) and C(11) in α-santonin confers any absorption in the 300 mμ region of the ultraviolet spectrum (Barton, Levisalles and Pinhey, 1962). These bonds therefore cannot be altered in the rearrangement, and the stereochemistry of santonin at C(6), C(7) and C(11) defines the stereochemistry of isophoto-α-santonic lactone at these centres.

The asymmetric centres at C(1) and C(10) are more difficult to define. Some chemical evidence formulates the

methyl group at C(10) as β(Barton, 1958) and assigns the α conformation to the hydrogen at C(1) (Barton, Levisalles and Pinhey, 1962). Conflicting evidence suggests that the hydrogen on C(1) and the hydroxyl on C(10) are cis and β (Huffman, 1960). Optical rotatory dispersion measurements indicate that the hydrogen on C(1) is β (Djerassi, Osiecki and Herz, 1957). Clearly some definitive evidence is needed.

A heavy atom derivative, bromodihydroisophoto-α-santonic lactone acetate, was prepared (Barton, Levisalles and Pinhey, 1962). This compound crystallised well and was suitable for x-ray structure analysis.

3. (2) EXPERIMENTAL

Rotation, oscillation, precession and moving film photographs were taken from a crystal rotated about the crystallographic axis c. Copper-K_α and Molybdenum-K_α radiation were employed, and cell dimensions were measured from these photographs. The space group was determined uniquely from the systematic halvings in the reflections.

The intensity data, consisting of the layer lines hk0....hk4, were collected by means of equi-inclination Weissenberg exposures, the multiple film technique being used to correlate strong and weak reflections (Robertson, 1943). For upper layer data, the film to film correlation factors given by Rossman (1956) were employed. A small

crystal was used, completely bathed in a uniform x-ray beam, and no corrections for absorption were applied. The intensities were corrected for Lorentz, polarization, and the rotation factors appropriate to upper layers (Tunell, 1939). The various layers were placed on the same relative scale by comparison with common reflections on $0k\ell$ and $h0\ell$ precession photographs. The absolute scale was obtained at a later stage by correlation with the final calculated structure amplitudes. 1022 reflections were indexed and 865 intensities measured.

The calculated density, assuming four molecules in the unit cell, is 1.526 g./ml. This is a typical value for this kind of compound, and the analysis proceeded on the basis of $Z = 4$. It was impracticable at a later date to measure the density, as the crystals had decomposed to a black resinous gum. This may be due to loss of bromine and subsequent polymerization.

3. (3) CRYSTAL DATA

$C_{17}H_{23}O_5Br$ $\quad M = 387.3$

Orthorhombic

$a = 11.05$ Å ± 0.02 Å

$b = 19.23$ Å ± 0.02 Å

$c = 7.93$ Å ± 0.03 Å

Volume of the unit cell $= 1685$ Å^3

For $Z = 4$, ρ(calculated) $= 1.526$ g./ml.

$F(000) = 800$

Absorption coefficient for x-rays (λ= 1.542) = 38.7 cm.$^{-1}$

Systematic absences: (h00) = 2n + 1

(0k0) = 2n + 1

(00ℓ) = 2n + 1

Space group $P2_12_12_1$ - D_2^4.

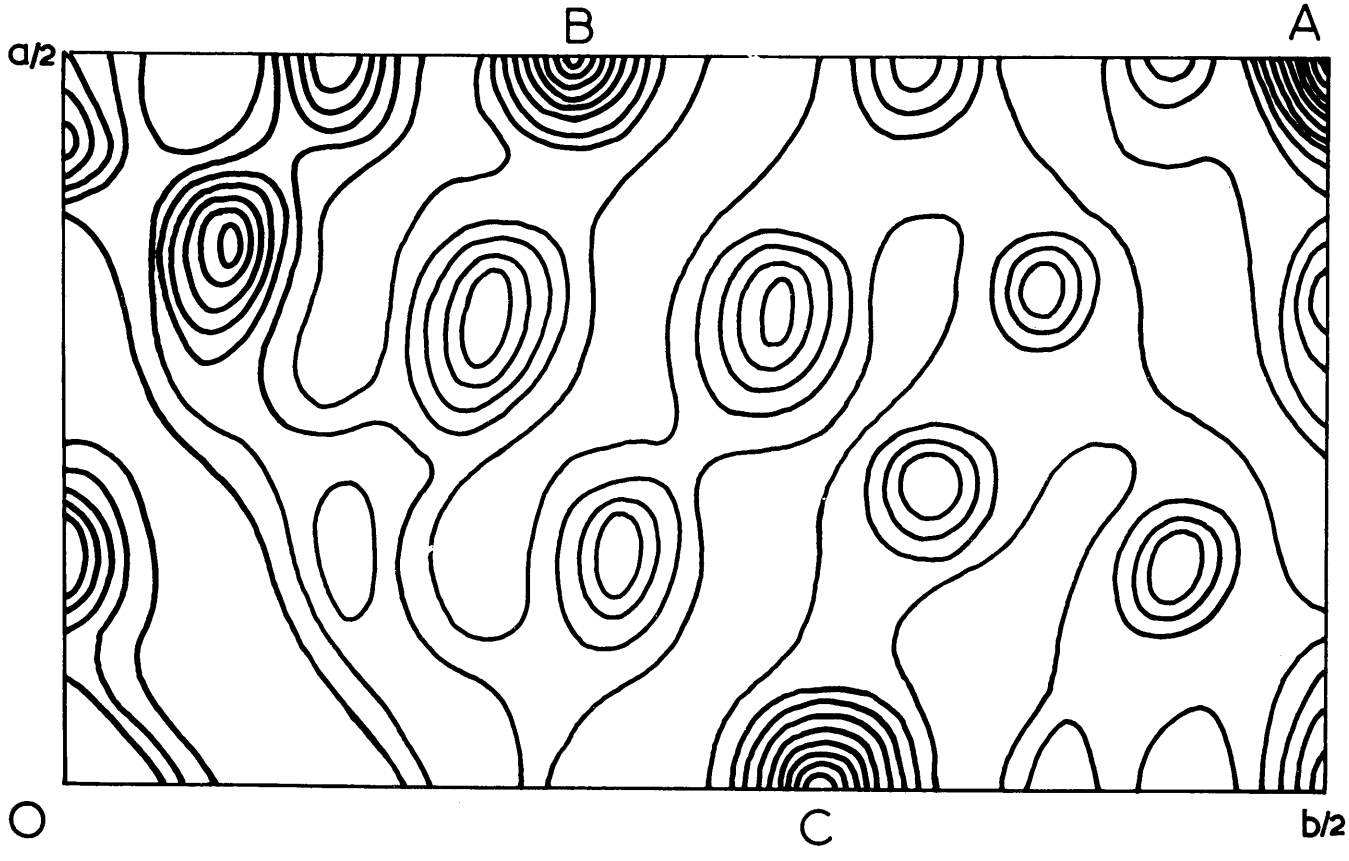
3. (4) LOCATION OF THE HEAVY ATOM

The heavy-atom vectors expected in the space group $P2_12_12_1$ where Z = 4 and there is one heavy atom in the asymmetric unit are listed below in tabular form

-	$\frac{1}{2}$-2x, -2y, $\frac{1}{2}$	$\frac{1}{2}$, $\frac{1}{2}$-2y, -2z	-2x, $\frac{1}{2}$, $\frac{1}{2}$-2z
$\frac{1}{2}$ + 2x, 2y, $\frac{1}{2}$	-	2x, $\frac{1}{2}$, $\frac{1}{2}$-2z	$\frac{1}{2}$, $\frac{1}{2}$ + 2y, -2z
$\frac{1}{2}$, $\frac{1}{2}$ + 2y, 2z	-2x, $\frac{1}{2}$, $\frac{1}{2}$ +2z	-	$\frac{1}{2}$-2x, 2y, $\frac{1}{2}$
2x, $\frac{1}{2}$, $\frac{1}{2}$ + 2z	$\frac{1}{2}$, $\frac{1}{2}$-2y, 2z	$\frac{1}{2}$ + 2x, -2y, $\frac{1}{2}$	-

The two-dimensional Patterson projections P(UV) and P(VW) were computed using 224 and 112 coefficients respectively; the resulting maps are shown in Figs. 9 and 10 respectively. Since the space group of the Patterson function in the orthorhombic system is Pmmm, it was not necessary to compute complete sections.

In the section projected down (001), there should be double weight peaks at $2x_{Br}$, $\frac{1}{2}$ on the line (U, $\frac{1}{2}$) and at $\frac{1}{2}$, $\frac{1}{2}$-$2y_{Br}$ on the line ($\frac{1}{2}$, V) with a single weight peak at


B
A
a/2
O
C
b/2

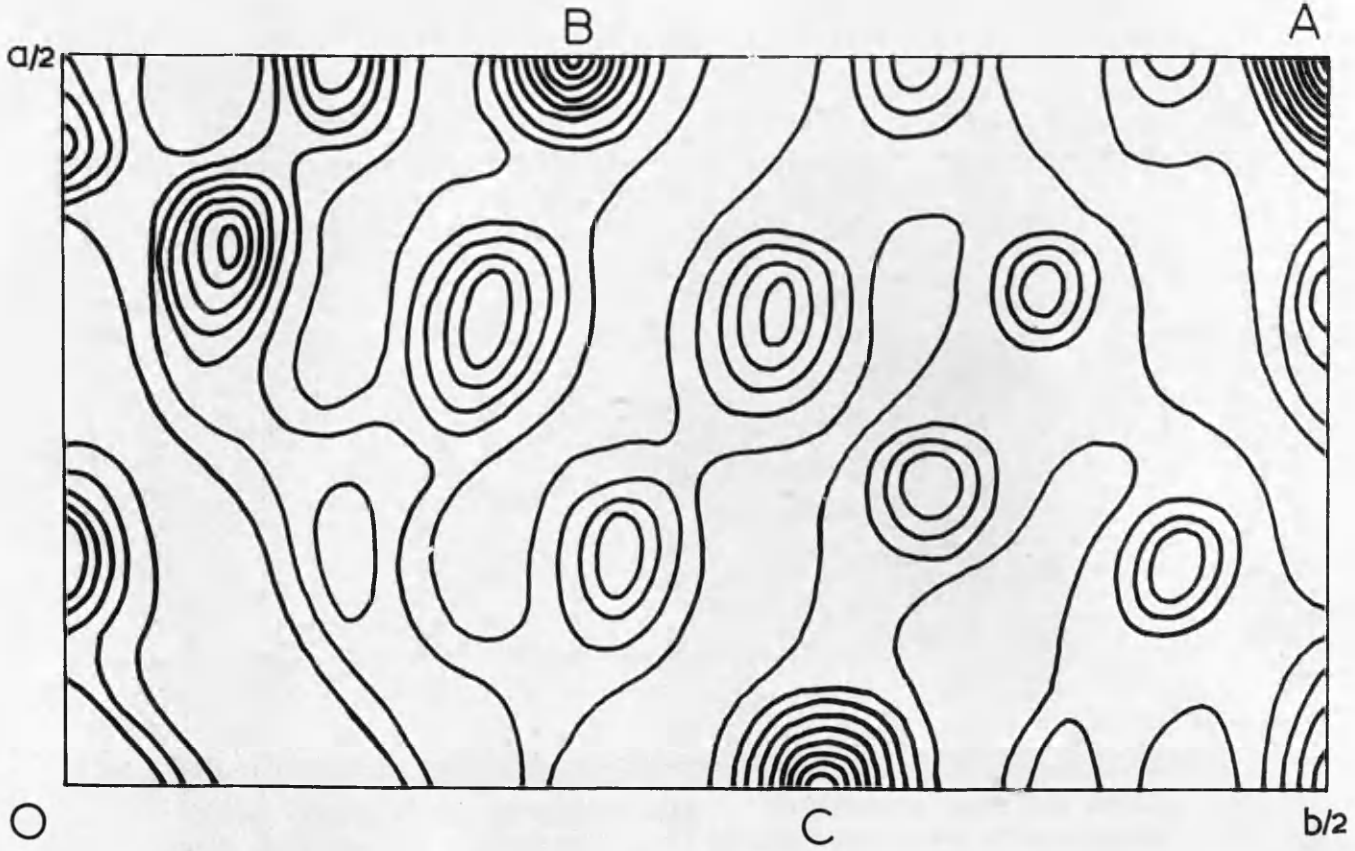


Fig. 9. Bromodihydroisophoto-α-santonic lactone acetate. (OOl) Patterson projection. Contours are at equal and arbitrary intervals. Br-Br vectors discussed in the text are marked A, B and C.

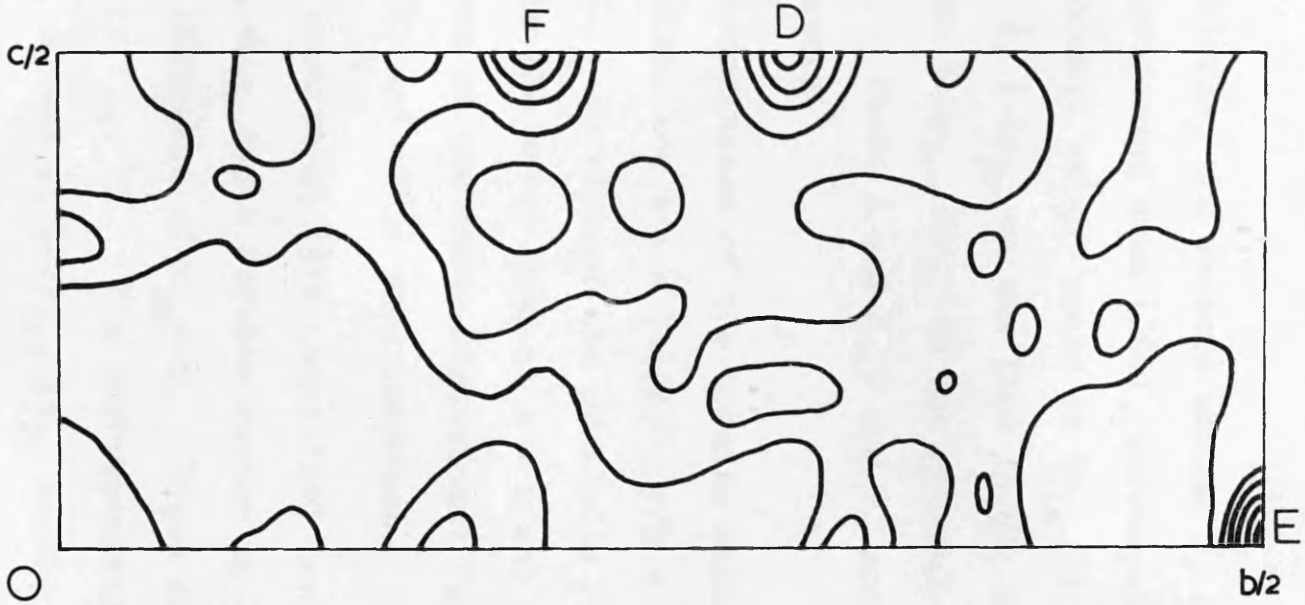


Fig. 10. Bromodihydroisophoto-α-santonic lactone acetate (100) Patterson projection. Contours are at equal and arbitrary intervals. Br-Br vectors discussed in the text are marked D, E and F.

$\frac{1}{2}-2x_{Br}$, $2y_{Br}$ in a general position. These vectors were clearly resolved, and are marked A, B and C respectively in Fig. 9.

Similarly, the vectors marked D, E and F in Fig.10, the section projected down (100), correspond to the anticipated double weight peaks at $2y_{Br}$, $\frac{1}{2}$ on the line $(V,\frac{1}{2})$ and at $\frac{1}{2}$, $\frac{1}{2}-2z_{Br}$ on the line $(\frac{1}{2},W)$, and the single weight peak at $\frac{1}{2}-2y_{Br}$, $2z_{Br}$ in the general position respectively. Peaks A and E, B and F, and C and D pertain to common vectors.

The coordinates of the bromine atom, calculated from these projections are x/a = 7.45/30, y/b = 9.18/60, z/c = 7.25/30. In view of the proximity of the x and z coordinates to the screw axes at $x = \frac{1}{4}$ and $z = \frac{1}{4}$, the three Harker sections of the three-dimensional Patterson synthesis at $(\frac{1}{2}VW)$, $(U\frac{1}{2}W)$ and $(UV\frac{1}{2})$ were computed.

The section at $(\frac{1}{2}VW)$ contained the expected vector at $\frac{1}{2}$, $\frac{1}{2}-2y_{Br}$, $2z_{Br}$ and a further vector at $2x_{Br}$, $\frac{1}{2}$, $\frac{1}{2}-2z_{Br}$ arising from the value of $x_{Br}=\sim\frac{1}{4}$. These are marked P and Q respectively in Fig. 11. The corresponding vectors at $\frac{1}{2}-2x_{Br}$, $2y_{Br}$, $\frac{1}{2}$, and $\frac{1}{2}$, $\frac{1}{2}-2y_{Br}$, $2z_{Br}$ in the section at $(UV\frac{1}{2})$ are marked R and S respectively in Fig. 12. The Harker section at $(U\frac{1}{2}W)$ contained only the vector $2x_{Br}$, $\frac{1}{2}$, $\frac{1}{2}-2z_{Br}$ marked T in Fig. 13.

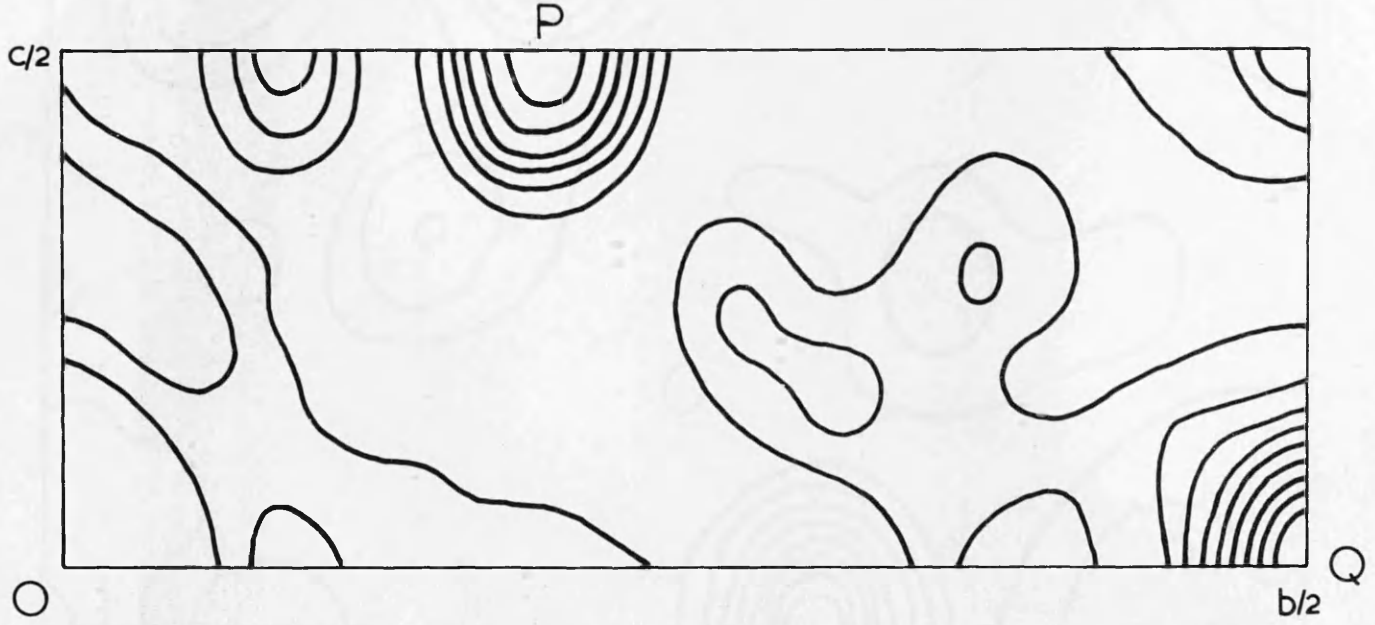


Fig. 11. Bromodihydroisophoto-α-santonic lactone acetate.
Harker section P($\frac{1}{2}$VW) of the 3D Patterson synthesis.
Contours are at equal and arbitrary intervals.
Br-Br vectors discussed in the text are marked P and Q.

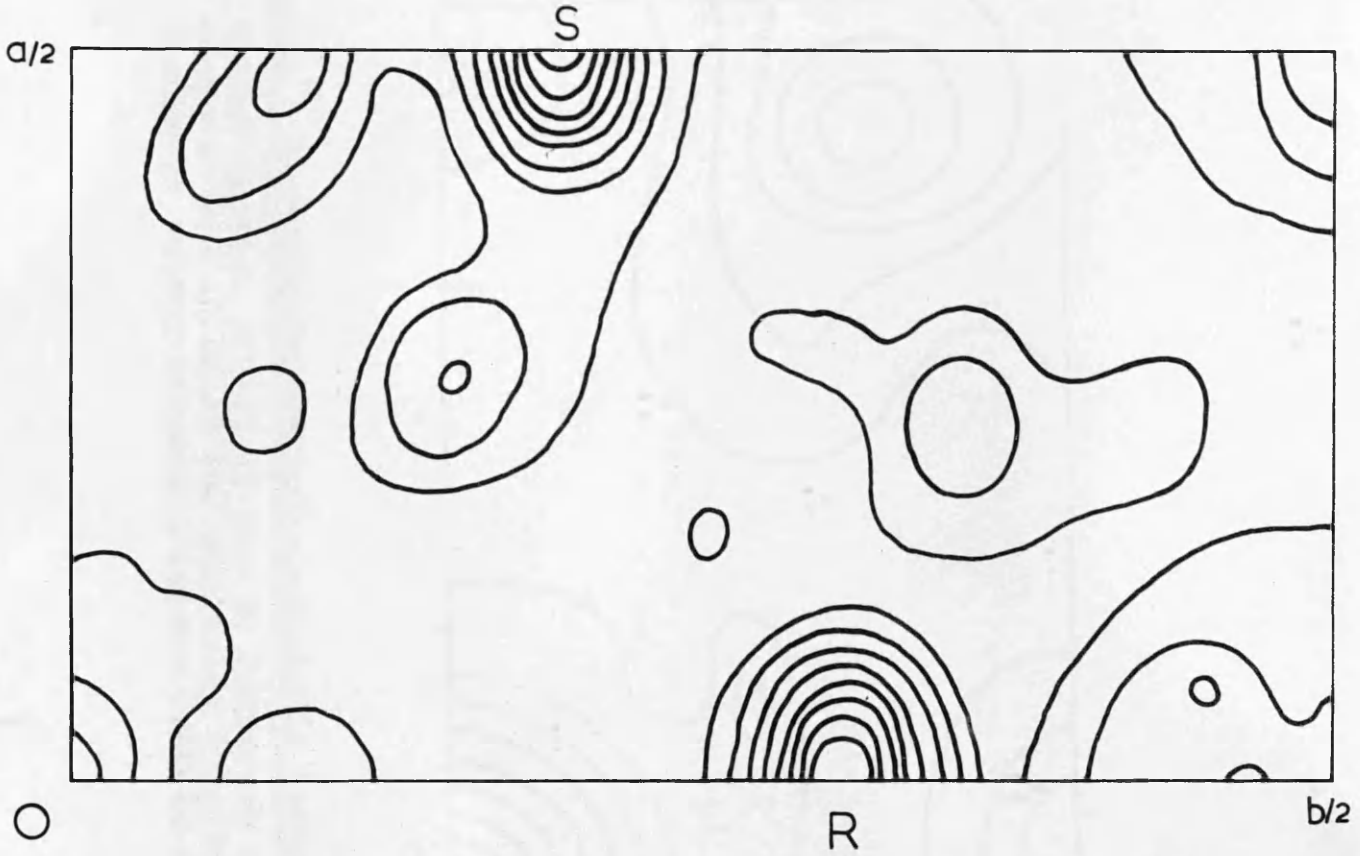


Fig. 12. Brepodihydroisophoto-α-santonic lactone acetate.
Harker section P(UV½) of the 3D Patterson synthesis.
Contours are at equal and arbitrary intervals.
Br-Br vectors discussed in the text are marked R and S.

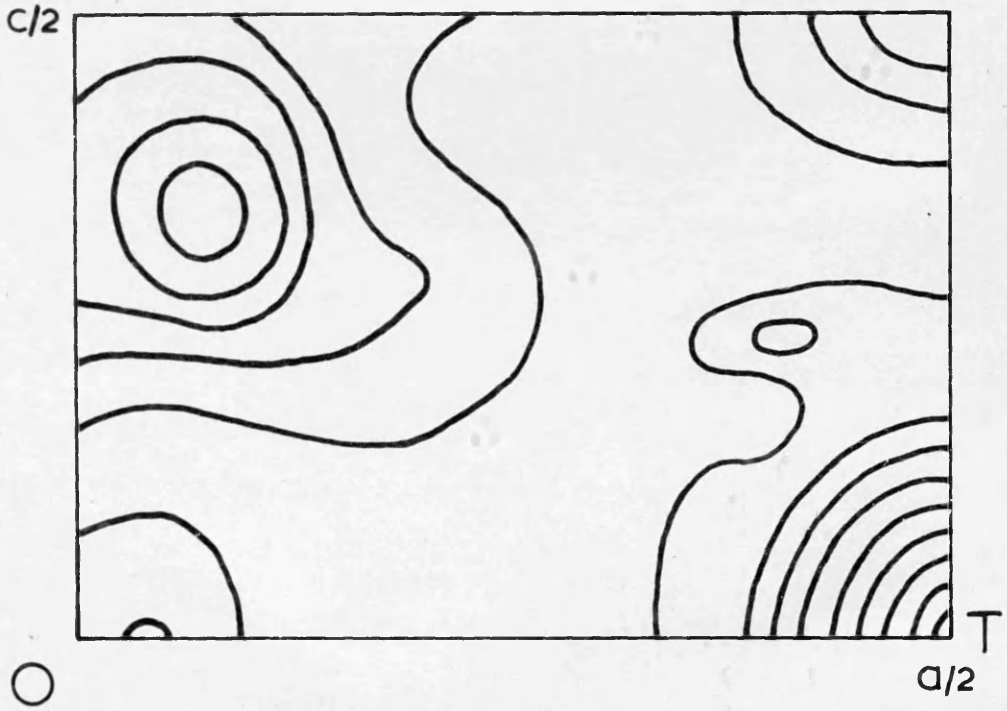


Fig.13. Bromodihydroisophoto-2-santonic lactone acetate.

Harker section P(U½W) of the 3D Patterson synthesis
Contours are at equal and arbitrary intervals.
The Br-Br vector discussed in the text is marked T.

Location of the bromine atom exactly on the special positions at $x = \frac{1}{4}$ and $z = \frac{1}{4}$ would result in completely spherical peaks centred on the mirror planes of the Harker sections. The elliptical nature of the peaks at P and Q showing elongation in the W direction and at R and S in the U direction confirmed that x_{Br} and z_{Br} were not exactly at $\frac{1}{4}$. Furthermore, in the Harker section at $(U\frac{1}{2}W)$, the major axis of the ellipse at T (Fig. 13) is in the W direction indicating that z_{Br} is further away from $z = \frac{1}{4}$ than x_{Br} from $x = \frac{1}{4}$.

All the vectors in both Patterson and Harker sections are consistent with one set of values for x_{Br}, y_{Br} and z_{Br}, and the coordinates obtained for the bromine atom were:-

$x/a = 0.242$ $\quad$ $y/b = 0.152$ $\quad$ $z/c = 0.233$

3. (5) SOLUTION OF THE STRUCTURE

The first set of structure factors, based on the heavy atom alone, gave a discrepancy of 45.1%. 159 structure factors with uncertain phase were rejected, and 863 coefficients included in the first three-dimensional Fourier synthesis.

The heavy atoms in the crystal are related to one another by higher symmetry than are the molecules as a whole,

in this case two centres of symmetry. The phases deduced by their contributions alone, in turn, introduce this symmetry into the calculated structure. Consequently, although peaks appeared in the Fourier synthesis at approximately the correct positions, they also occurred at additional positions, related to the first by two spurious mirror planes.

It appeared to be impossible to unravel the structure from its three interpenetrating "ghosts", and recourse was taken to assigning coordinates on the basis of peak heights of alternative sites related by spurious symmetry; three were adjudged to predominate significantly over their respective "ghosts", and were included in a second cycle of structure factor and Fourier calculation. This approach was pursued for five cycles in all, and the results are shown in tabular form in Table 12.

No sensible chemical structure or fragment of structure could be seen after the fifth cycle and it was obvious that the approach was unprofitable.

The x coordinate of the bromine atom had moved slightly further away from the screw axis, and it was decided to compute a sixth Fourier with phases determined solely by the bromine atom in its new position, with a view firstly, to checking the positions to which atoms

TABLE 12.

Bromodihydroisophoto -d- Santonic Lactone Acetate

Course of the Structure Determination

Operation	Atoms included	R(%)
2D Patterson syntheses		
3D Harker syntheses		
1st 3D Fourier synthesis	Br	45.1
2nd " " "	Br + 3 (C)	42.0
3rd " " "	Br + 8 (C)	38.9
4th " " "	Br + 11 (C)	37.7
5th " " "	Br + 13 (C)	36.9
6th " " "	Br	43.7
7th " " "	Br + 5 (C)	40.5
8th " " "	Br + 12 (C)	36.6
9th " " "	Br + 14(C) + 3(O)	32.3
10th " " "	Br + 16(C) + 4(O)	29.2
11th " " "	Br + 16(C) + 5(O)	24.8

had been allocated, and secondly, perhaps, to disclose new atomic sites. B_0 was raised from 4.4 $\mathring{A}^2$ to 4.9 $\mathring{A}^2$.

The residual of 43.7% showed a drop of 1.4% compared with the first structure factor calculation, but more important was the appearance, in the Fourier map in an unambiguous position, of the atom in the five membered carbocyclic ring which is covalently bonded to the bromine atom. The presence of this atom made it possible to disentangle the five membered carbocyclic ring with some confidence, since peaks chosen were consistently higher than their "ghosts". Where an option had been present, comparison with sites determined previously showed that a different choice was made in some cases.

These five carbon atoms were included in a seventh structure factor calculation and the electron-density map revealed the seven-membered carbocyclic ring and its two substituents at C(10). The lactone ring was also nebulously defined, but no sites were sufficiently well resolved in this latter ring to permit inclusion of any of its constituent atoms in the eighth structure factor calculation.

Successive cycles of structure factor and Fourier calculations progressively revealed more and more atoms until the tenth Fourier map disclosed the complete structure. The course of the structure determination appears in Table 12.

Correspondence with Professor Barton at this stage indicated that the stereochemistry assigned at all the asymmetric centres was acceptable with the exception of the conformation of the methyl group C(13) at C(11). The controversial C(13) was omitted in an eleventh structure factor calculation, and the atom reappeared in exactly the same position in the resulting Fourier map. Very few areas of spurious electron density appeared on the map and the structure was judged to be firmly established.

3. (6) REFINEMENT

Refinement was carried out by F_o and F_c syntheses in the initial period, and was initiated at the stage of the tenth cycle of structure factor and Fourier calculation. The back-shift corrections, compensating for termination of series effects were applied to the coordinates from the F_o synthesis, giving an improved structure. Isotropic temperature factors were assigned individually; in general there was a slight increase in B_0.

This method of refinement was pursued for four cycles and was accompanied by a drop in the discrepancy to 20.3% from 29.24%. The small improvement due to the fourth cycle indicated that refinement of atomic coordinates by this method was practically complete. The course of the refinement appears in Table 13.

TABLE 13.

Bromodihydroisophoto -α- Santonic Lactone Acetate

Course of Refinement

Operation	Atoms included	R(%)	$\sum \omega \Delta^2$
10th 3D Fourier synthesis (F_o and F_c)	Br + 16(C)+4(O)	29.2	-
11th " " "	Br + 16(C)+5(O)	24.8	-
12th " " "	Br + 17(C)+5(O)	21.1	-
13th " " "	Br + 17(C)+5(O)	20.3	-
1st Least squares cycle	Br + 17(C)+5(O)	20.2	250
2nd " " "	Br + 17(C)+5(O)	17.2	152
3rd " " "	Br + 17(C)+5(O)	15.8	125
4th " " "	Br + 17(C)+5(O)	15.1	110
5th " " "	Br + 17(C)+5(O)	14.6	104
6th " " "	Br + 17(C)+5(O)	21.4	596
7th " " "	Br + 17(C)+5(O)	16.4	322
8th " " "	Br + 17(C)+5(O)	15.0	298
9th " " "	Br + 17(C)+5(O)	14.8	224
10th " " "	Br + 17(C)+5(O)	14.0	203
11th " " "	Br + 17(C)+5(O)	14.3	295
12th " " "	Br + 17(C)+5(O)	13.7	224
13th " " "	Br + 17(C)+5(O)	12.9	218
14th 3D Fourier synthesis (F_o)	Br + 17(C)+5(O)	12.9	-

Refinement was continued by the method of least squares, again only employing the block diagonal elements of the matrix of normal equations. The weighting system was the one used in refining hunterburnine β-methiodide (see Chapter 2. (6)) and the value of F^* for cycles one to five was 15, and 32 for the remainder. Half-scale scattering factors were used for all atoms and half-shift corrections were employed throughout. The location and contribution of hydrogen atoms were not considered.

After five cycles, bond length C(7)-C(11) was shorter than that predicted by theory and C(10)-O(21) abnormally long. Positional parameters of all four atoms were adjusted to give more reasonable bond lengths. Using the relationships $\beta_{11} = a^* B_{11}/4$ and $b_{11} = 1.4427\beta_{11}$ (Rossman, Jacobson, Hirshfield and Lipscomb, 1959), values of B_{11}, B_{22} and B_{33}, the thermal parameters parallel to each principal axis were obtained for all the atoms in the structure. These were averaged for each atom, and the resulting isotropic temperature factors employed in the sixth cycle of least squares. The rise in discrepancy of 6.8% is a measure of the degree of anisotropy of the structure.

After a further four cycles of least squares had been completed, the C(7)-C(11) bond length was again uncommonly short. After readjustment of the positional parameters of C(7) and C(11), refinement was continued using

the anisotropic thermal parameters obtained from the previous cycle.

Refinement was terminated after a further three cycles. The final value of R was 12.85% and this was increased to 13.83% on the inclusion of unobserved reflections.

Throughout the analysis, theoretical atomic scattering factors due to Berghius et al. (1935) for carbon and oxygen were used, and those due to Thomas and Fermi (1935) for bromine.

3. (7) RESULTS

On the basis of the final phases, a final three-dimensional Fourier map was computed and is represented in Fig. 14 by means of superimposed contour sections drawn parallel to (001). The corresponding atomic arrangement appears in Fig. 15. The arrangement of the molecules in the crystal as viewed in projection along the a axis is shown in Fig. 16 and along the c axis in Fig. 17.

The final values of $|F_o|$, $|F_c|$ and α are given in Table 14, final positional parameters in Table 15, and final anisotropic thermal parameters in Table 16. Table 17 contains intramolecular bonded distances and Table 18 the interbond angles. Intramolecular non-bonded distances < 4 Å are recorded in Table 19 and inter-molecular contacts < 4 Å

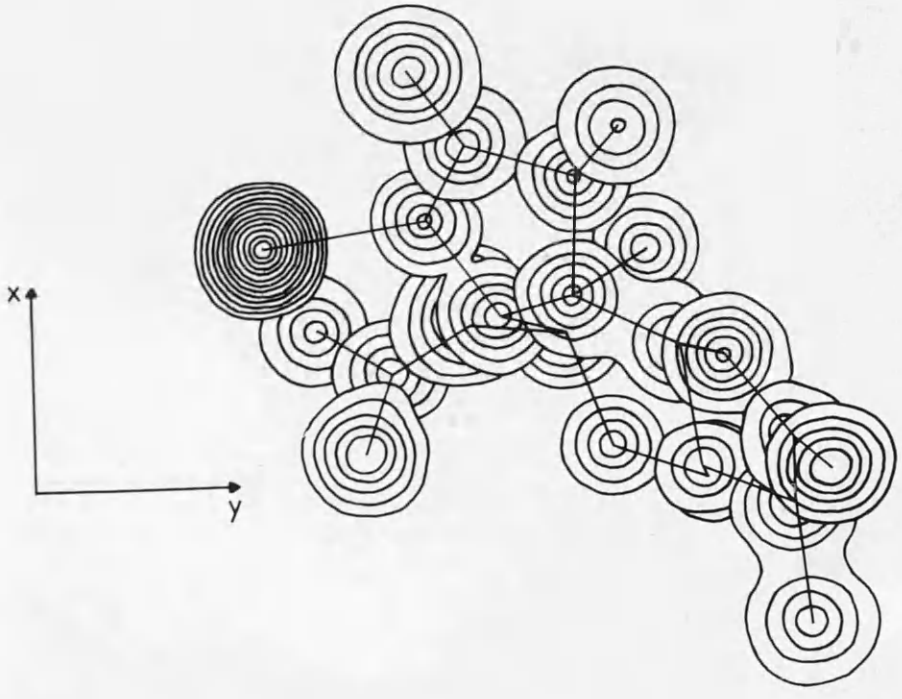


Fig. 14. Progodihydroisophoto-α-santonic lactone acetate.

Superimposed contoured sections of the final 3D Fourier synthesis covering the region of one molecule. The sections are drawn parallel to (001). Contours are drawn at intervals of 1 electron/Å^3 with the exception of the bromine atom for which intervals are at 3 electrons/Å^3.

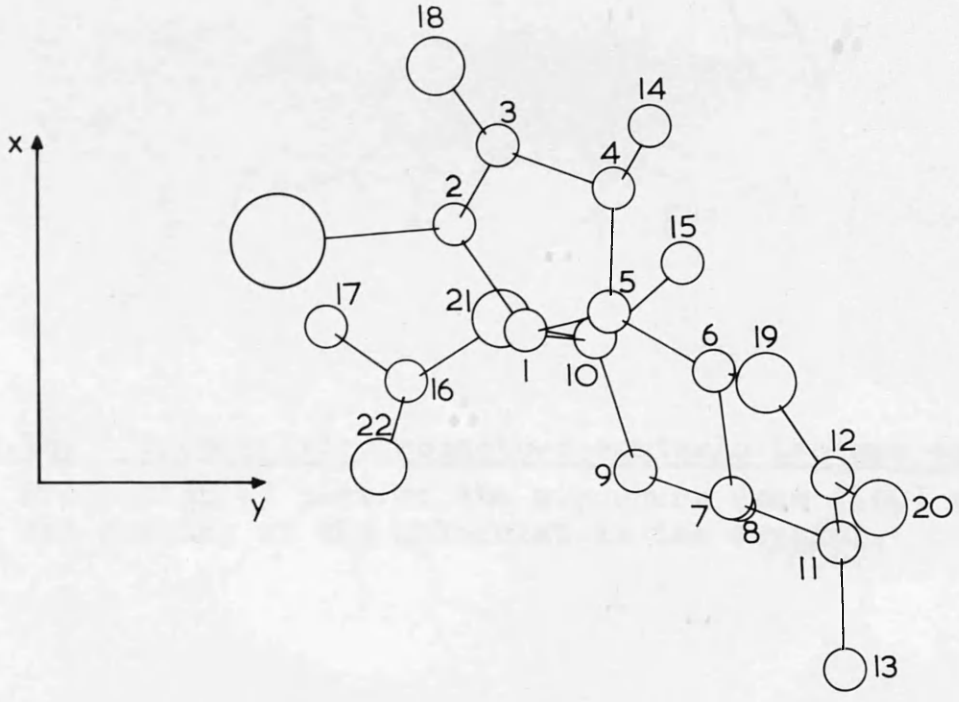


Fig. 15. Bromodihydroisophoto-c-santonic lactone acetate
Atomic arrangement corresponding to Fig.14.

Fig.16. Bromodihydroisophoto-α-santonic lactone acetate.

Projection of part of the structure down (100) showing the packing of the molecules in the crystal.

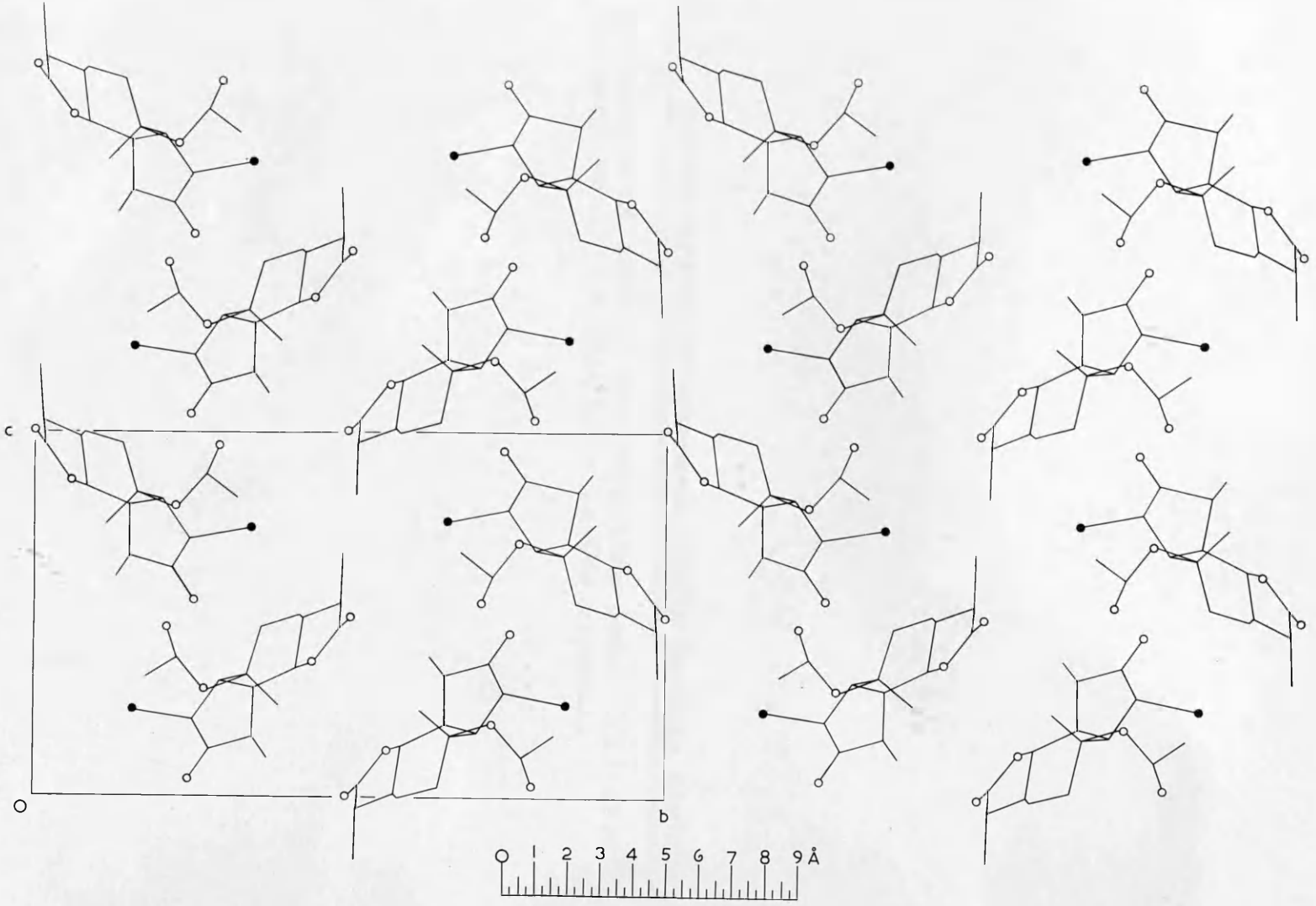

c
O
b
O 1 2 3 4 5 6 7 8 9 Å

Fig.17. Bromodihydroisophoto-α-santonin lactone acetate.

Projection of part of the structure down (001) showing the packing of the molecules in the crystal.

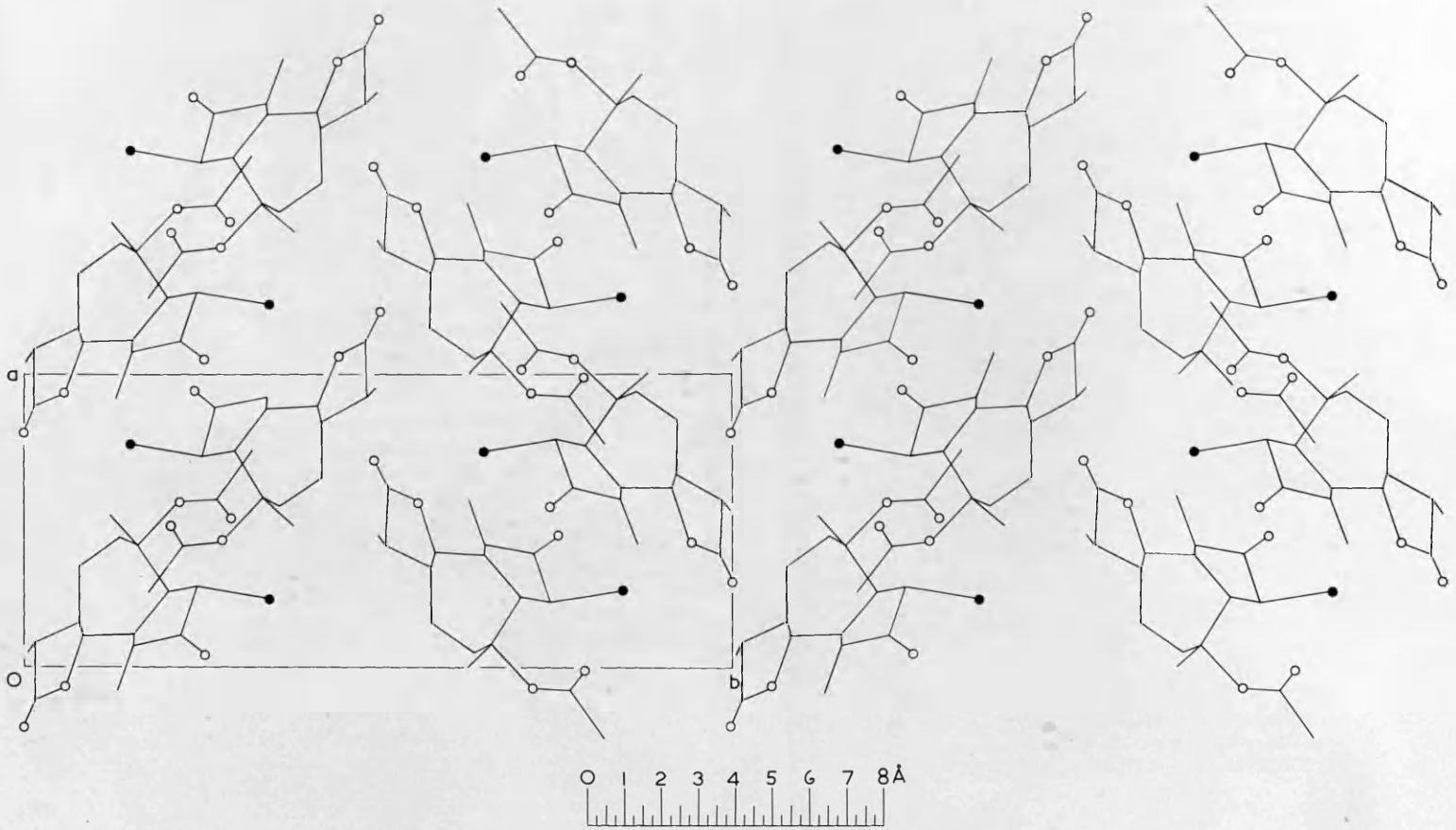

a
O
b
0 1 2 3 4 5 6 7 8Å

Table 14. Bromodihydroisophoto-α-santonic lactone acetate.

Final measured and calculated values of the structure factors. Unobserved terms have been omitted.

h	k	ℓ	$\|F_o\|$	$\|F_c\|$	ω°
0	1	3	28	33	270
0	2	0	75	100	180
		1	12	23	0
		2	21	18	0
		3	76	90	180
		4	31	35	180
		5	59	67	90
0	3	1	19	16	270
		2	14	14	90
		3	16	15	270
0	4	0	21	15	180
		1	40	44	180
		2	50	58	0
		3	36	50	0
		4	15	16	180
0	5	1	30	31	90
		2	5	6	90
		3	37	33	90
		4	10	19	90
0	6	0	81	92	0
		1	66	71	180
		2	77	88	180
		3	51	60	0
		4	50	54	0
0	7	1	17	17	90
		2	10	11	270
		3	9	5	270
		4	24	22	90
0	8	0	38	45	0
		1	91	87	0
		2	9	9	0
		3	75	72	180
		4	16	16	0
0	9	1	36	38	90
		2	25	19	90
		3	4	5	90
0	10	0	72	69	180
		2	53	48	0
		3	23	20	0
		4	19	14	180
0	11	1	38	32	270
		2	10	9	90
		3	10	11	90
0	12	0	64	58	0
		1	50	49	180
		2	42	42	180
		3	46	42	0
		4	9	11	0
0	13	1	22	20	90
		2	7	7	90
		3	10	8	90
		4	11	15	90
0	14	0	70	65	0
		1	25	23	0
		2	14	15	180
		3	43	45	180
0	15	1	8	9	90
		2	5	2	270
		3	12	13	270
0	16	0	42	39	180
		2	21	23	0
		3	8	11	180
0	17	1	15	15	90
		2	12	10	270
		3	9	11	90
0	18	0	8	7	0
		1	46	51	180
		2	7	7	0
		3	21	26	0
0	19	3	5	1	270
0	20	0	23	20	0
		1	12	12	0
		2	27	24	180
		3	7	9	180
0	21	1	8	7	90
		3	4	0	270
0	22	0	24	21	180
		1	18	12	0
		2	16	12	0
		3	4	7	180
0	23	1	3	2	90
0	24	1	11	9	180
1	0	2	26	38	0
		3	11	11	90
		4	7	9	180
1	1	0	11	7	270
		1	112	147	70
		2	64	88	274
		3	45	66	267
		4	29	39	81
1	2	0	52	63	90
		1	35	30	57
		2	64	60	179
		3	52	54	98
		4	29	32	210
1	3	0	12	27	270
		1	82	65	8
		2	125	131	90
		3	12	11	138
		4	52	54	275
1	4	0	88	88	90
		1	64	70	287
		2	23	22	104
		3	28	30	260
		4	28	31	314
1	5	0	48	37	90
		1	117	116	294
		2	28	20	82
		3	17	18	47
		4	5	7	93
1	6	0	18	13	90
		1	24	26	275
		2	6	5	133
		3	43	40	88
		4	32	32	204
1	7	0	98	106	90
		1	37	42	138
		2	78	74	277
		3	31	29	221
		4	37	43	86
1	8	0	21	19	90
		1	31	27	282
		2	39	25	94
		3	19	22	36

h	k	ℓ	$\|F_o\|$	$\|F_c\|$	ω°
		4	58	58	270
		5	50	60	72
		6	58	66	81
		7	37	35	283
		8	36	40	285
1	10	0	22	23	270
		1	19	16	174
		2	13	11	137
		3	13	12	149
1	11	1	70	72	281
		2	28	26	122
		3	25	20	116
		4	19	21	223
1	12	0	10	9	270
		1	17	16	186
		2	8	6	28
		3	16	19	113
		4	15	9	213
1	13	0	48	43	90
		1	7	9	35
		2	47	47	265
		3	11	12	98
		4	30	39	53
1	14	0	13	12	90
		1	7	6	153
		2	17	17	34
		3	5	4	43
1	15	0	16	17	270
		1	45	42	88
		2	23	21	108
		3	27	25	274
1	16	0	16	14	90
		1	7	6	10
		2	25	21	187
		3	8	7	51
1	17	0	22	21	270
		1	22	20	275
		2	26	18	93
		3	13	15	279
1	18	0	10	10	90
		1	11	10	272
		2	13	11	75
1	19	0	22	17	90
		1	32	22	268
		2	29	23	269
		3	6	4	211
		4	3	2	348
1	20	0	33	26	92
		1	32	18	267
		2	4	2	320
1	21	0	14	13	270
		1	9	9	246
2	0	0	60	78	180
		1	82	87	90
		2	91	107	0
		3	34	45	0
		4	12	10	6
2	1	0	34	45	0
		1	12	10	6
		2	26	23	301
		3	26	22	155
		4	18	14	270
2	2	0	119	121	0
		1	112	131	218
		2	92	101	186
		3	58	63	359
		4	14	13	325
2	3	0	10	16	0
		1	67	61	191
		2	8	7	12
		3	8	20	120
		4	36	34	106
2	4	0	116	137	0
		1	41	40	90
		2	37	35	165
		3	40	32	171
		4	37	44	354
2	5	0	21	20	0
		1	44	43	94
		2	25	21	321
		3	34	32	204
		4	13	11	90
2	6	0	75	93	180
		1	55	46	35
		2	54	61	332
		3	45	46	179
		4	24	19	201
2	7	0	41	35	0
		1	35	25	289
		2	58	65	316
2	8	0	8	4	0
		1	68	74	147
		2	20	17	286
		3	9	7	74
		4	19	18	279
2	9	0	47	52	180
		1	18	18	151
		2	16	18	271
		3	17	14	74
		4	9	8	160
2	10	0	75	81	0
		1	20	18	17
		2	71	69	184
		3	14	11	184
		4	35	27	8
2	11	0	17	16	55
		1	15	17	134
		2	15	17	134
		3	7	10	274
		4	15	20	40
2	12	0	24	26	180
		1	45	50	352
		2	18	18	14
		3	27	27	164
		4	17	16	171
2	13	0	17	15	180
		1	20	20	91
		2	9	8	218
		3	9	7	84
		4	15	14	246
2	14	0	10	8	180
		1	33	38	171
		2	17	16	347

h	k	ℓ	$\|F_o\|$	$\|F_c\|$	ω°
2	14	3	40	36	357
		4	10	9	263
		5	7	8	329
		6	3	8	183
2	15	0	45	44	0
		1	19	19	209
		2	27	26	176
		3	7	4	31
		4	3	11	3
2	17	0	5	4	180
		1	7	6	150
		2	4	6	181
		3	8	7	280
		4	9	8	73
2	18	0	13	12	180
		1	26	25	14
		2	9	9	193
		3	17	20	186
2	19	0	4	5	0
		1	8	8	87
		2	6	7	160
2	20	0	35	28	180
		1	14	12	196
		2	23	21	344
		3	6	9	11
		4	21	16	299
2	21	0	8	5	270
2	22	0	21	16	0
		1	11	8	159
		2	14	14	195
		3	8	10	1
3	0	1	32	32	90
		2	16	16	180
		3	16	11	90
3	1	0	66	77	270
		1	76	95	269
		2	51	53	67
		3	55	56	96
		4	21	16	299
3	2	0	8	5	270
		1	49	55	324
		2	28	32	194
		3	13	12	114
		4	24	24	216
3	3	0	58	69	90
		1	39	40	211
		2	77	86	266
		3	25	22	207
		4	33	34	101
3	4	0	30	35	90
		1	26	22	283
		2	26	27	353
		3	29	27	64
		4	8	6	128
3	5	0	16	18	90
		1	83	97	56
		2	28	22	63
		3	31	40	277
		4	18	15	319
3	6	0	36	36	270
		1	35	36	294
		2	48	44	214
		3	19	10	258
		4	16	15	275
3	7	0	80	81	270
		1	30	35	257
		2	46	53	111
		3	15	14	141
		4	46	42	251
3	8	0	7	5	90
		1	37	28	218
		2	21	26	98
		3	12	11	199
		4	8	8	84
3	9	0	78	76	90
		1	77	59	277
		2	22	25	290
		3	28	26	107
		4	31	30	87
3	10	0	11	7	90
		1	26	29	283
		2	19	19	55
		3	17	15	76
		4	13	11	291
3	11	0	25	19	90
		1	55	54	98
		2	16	17	290
		3	16	13	266
		4	15	16	32
3	12	1	7	8	117
		2	22	25	299
		3	50	46	270
		4	24	25	263
3	13	0	4	4	86
		1	21	16	0
		2	8	10	1
		3	10	11	54
		4	21	22	105
3	14	0	30	23	90
		1	21	17	102
		2	19	19	285
		3	9	13	267
3	15	0	26	17	90
		1	8	7	80
		2	32	44	82
		3	19	18	280
		4	27	27	264
3	16	0	18	21	270
		1	28	18	206
		2	8	8	160
		3	23	19	167
		4	11	12	348
3	17	0	29	24	90
		1	44	38	266
		2	16	14	140
		3	50	51	91
3	18	0	18	13	111
		1	25	26	308
		2	20	18	62
		3	10	14	220
3	19	0	17	15	90
		1	10	2	246
3	20	0	89	107	0
		1	13	10	90
		2	73	82	180
		3	11	11	90
		4	61	66	0

h	k	ℓ	$\|F_o\|$	$\|F_c\|$	ω°
4	1	0	27	27	180
		1	36	36	231
		2	14	14	211
		3	21	19	307
		4	18	13	66
4	2	0	2	2	180
		1	48	63	347
		2	6	6	231
		3	64	60	100
		4	30	25	191
4	3	0	26	24	0
		1	45	44	303
		2	13	12	40
		3	32	34	198
4	4	0	34	39	180
		1	69	84	183
		2	32	35	1
		3	22	21	359
4	5	0	25	17	182
		1	13	14	180
		2	18	19	186
		3	15	14	215
		4	31	26	44
4	6	0	13	12	139
		1	58	69	0
		2	34	35	220
		3	42	48	188
		4	24	30	347
4	7	0	29	25	338
		1	9	6	180
		2	25	23	149
		3	20	21	342
		4	12	11	195
4	8	0	32	35	0
		1	52	58	11
		2	6	9	129
		3	47	46	182
		4	19	23	30
4	9	0	21	20	0
		1	25	24	35
		2	22	19	107
		3	9	8	357
		4	12	12	310
4	10	0	33	35	180
		1	9	10	234
		2	47	52	345
		3	12	14	5
		4	22	22	202
4	11	0	9	8	180
		1	13	15	201
		2	27	25	358
		3	12	13	175
		4	11	11	191
4	12	0	38	35	0
		1	35	33	185
		2	38	37	180
		3	29	27	351
		4	9	9	338
4	13	1	19	22	93
		2	14	12	232
		3	20	19	0
		4	28	28	354
4	14	0	27	25	185
		1	12	11	7
		2	5	3	0
		3	26	19	180
		4	7	7	75
4	15	0	23	20	345
		1	14	17	202
		2	5	2	0
		3	1	7	8
		4	2	8	9
4	16	0	3	5	114
		1	9	9	180
		2	32	28	196
		3	14	16	353
		4	4	5	180
		5	7	7	183
4	17	0	19	20	0
		1	8	9	22
		2	17	14	187
		3	8	8	186
4	18	0	4	4	0
		1	21	19	90
		2	21	16	180
		3	39	45	191
		4	9	7	1
4	19	0	8	10	270
		1	8	7	180
		2	7	8	180
		3	42	34	90
		4	10	12	180
4	20	0	54	51	90
		1	53	61	82
		2	24	26	258
		3	36	36	266
		4	48	50	90
		5	36	34	167
4	21	0	14	13	264
		1	10	11	54
		2	89	89	270
		3	18	16	62
		4	18	21	270
4	22	0	28	18	206
		1	8	8	160
		2	23	19	167
		3	11	12	348
		4	29	24	90
		5	44	38	266
		6	16	14	140
4	23	0	50	51	91
		1	18	13	111
		2	18	20	62
		3	10	14	220
4	24	0	17	15	90
		1	10	2	246
		2	89	107	0
		3	13	10	90
		4	73	82	180
		5	11	11	90
		6	5	6	90

h	k	ℓ	$\|F_o\|$	$\|F_c\|$	ω°
5	0	1	14	14	90
		2	4	5	180
		3	11	10	270
		4	7	8	0
5	1	0	18	19	270
		1	21	24	282
		2	28	30	72
		3	33	33	86
		4	9	9	302
5	2	0	9	8	270
		1	8	8	252
		2	15	14	121
		3	13	14	104
		4	4	9	279
5	3	0	57	56	90
		1	18	15	335
		2	18	24	273
		3	13	13	99
		4	16	14	62
5	4	0	37	43	90
		1	15	15	268
		2	9	9	189
		3	4	8	214
5	5	0	11	10	270
		1	34	32	89
		2	5	6	107
		3	40	40	273
5	6	0	4	5	90
		1	13	13	215
		2	12	15	203
		3	6	6	327
		4	17	8	191
5	7	0	32	35	270
		1	25	23	231
		2	20	19	74
		3	7	5	91
5	8	0	14	15	270
		1	8	5	330
		2	20	17	80
		3	10	10	96
		4	12	12	284
5	9	0	6	6	270
		1	20	18	279
		2	12	10	316
		3	17	19	92
		4	19	16	270
5	10	0	5	5	90
		1	4	4	297
		2	4	5	328
5	11	0	21	15	12
		1	26	24	90
		2	7	8	222
		3	32	37	265
5	12	0	10	8	90
		1	18	13	51
		2	8	4	196
		3	8	5	347
5	13	0	31	31	270
		1	18	14	103
		2	5	2	304
		3	12	10	270
		4	3	6	189
5	14	0	8	9	90
		1	22	19	288
		2	3	6	77
		3	5	5	96
5	15	0	24	20	90
		1	13	11	94
		2	13	10	256
		3	9	10	269
5	16	0	10	10	90
		1	7	8	346
		2	8	9	45
		3	5	6	114
		4	2	5	180
		5	21	26	180
5	17	0	20	22	178
		1	3	5	224
		2	11	10	25
		3	23	23	90
		4	21	23	180
5	18	0	2	4	90
		1	21	20	330
		2	41	39	17
		3	5	8	273
5	19	0	7	4	5
		1	4	7	297
		2	8	8	159
		3	21	19	180
		4	39	45	191
5	20	0	12	13	291
		1	16	16	19
		2	4	9	10
		3	12	14	180
		4	17	19	167
5	21	0	14	15	23
		1	7	8	145
		2	22	22	136
5	22	0	40	41	0
		1	21	19	0
		2	39	39	181
		3	5	4	280
5	23	0	25	27	0
		1	6	7	158
		2	9	6	230
		3	4	8	20
6	0	1	24	23	358
		2	21	23	313
		3	12	11	179
		4	15	14	213
6	1	0	20	19	180
		1	10	6	0
		2	16	16	166
		3	2	6	253
		4	8	8	69
6	2	0	34	36	13
		1	22	18	236
		2	14	13	24
		3	24	23	3
		4	13	14	172
6	3	0	13	13	180
		1	6	5	149
		2	11	9	161
		3	19	19	145
		4	28	26	170
6	4	0	5	4	159
		1	21	22	7
		2	10	6	0
		3	16	16	166
		4	5	6	253
		5	4	8	69
6	5	0	34	36	13
		1	22	18	236
		2	14	13	24
		3	24	23	3
		4	13	14	172
6	6	0	13	13	180
		1	6	5	149
		2	11	9	161
		3	19	19	145
		4	28	26	170
6	7	0	5	4	159
		1	21	22	7
		2	10	6	0
		3	16	16	166
		4	5	6	253
		5	4	8	69
6	8	0	34	36	13
		1	22	18	236
		2	14	13	24
		3	24	23	3
		4	13	14	172
6	9	0	13	13	180
		1	6	5	149
		2	11	9	161
		3	19	19	145
		4	28	26	170
6	10	0	5	4	159
		1	21	22	7
		2	10	6	0
		3	16	16	166
		4	5	6	253
		5	4	8	69
6	11	0	34	36	13
		1	22	18	236
		2	14	13	24
		3	24	23	3
		4	13	14	172
6	12	0	13	13	180
		1	6	5	149
		2	11	9	161
		3	19	19	145
		4	28	26	170
6	13	0	5	4	159
		1	21	22	7
		2	10	6	0
		3	16	16	166
		4	5	6	253
		5	4	8	69
6	14	0	34	36	13
		1	22	18	236
		2	14	13	24
		3	24	23	3
		4	13	14	172
6	15	0	13	13	180
		1	6	5	149
		2	11	9	161
		3	19	19	145
		4	28	26	170
6	16	0	5	4	159
		1	21	22	7
		2	10	6	0
		3	16	16	166
		4	5	6	253
		5	4	8	69
6	17	0	34	36	13
		1	22	18	236
		2	14	13	24
		3	24	23	3
		4	13	14	172
6	18	0	13	13	180
		1	6	5	149
		2	11	9	161
		3	19	19	145
		4	28	26	170
6	19	0	5	4	159
		1	21	22	7
		2	10	6	0
		3	16	16	166
		4	5	6	253
		5	4	8	69
6	20	0	34	36	13
		1	22	18	236
		2	14	13	24
		3	24	23	3
		4	13	14	172
6	21	0	13	13	180
		1	6	5	149
		2	11	9	161
		3	19	19	145
		4	28	26	170
6	22	0	5	4	159
		1	21	22	7
		2	10	6	0
		3	16	16	166
		4	5	6	253
		5	4	8	69
6	23	0	34	36	13
		1	22	18	236
		2	14	13	24
		3	24	23	3
		4	13	14	172
6	24	0	13	13	180
		1	6	5	149
		2	11	9	161
		3	19	19	145
		4	28	26	170
6	25	0	5	4	159
		1	21	22	7
		2	10	6	0
		3	16	16	166

TABLE 15.

Bromodihydroisophoto -α- Santonic Lactone Acetate

Final Atomic Coordinates

Atom	x/a	y/b	z/c
C(1)	-0.1819	0.2011	0.2631
C(2)	-0.2895	0.2463	0.2763
C(3)	-0.3729	0.2225	0.1280
C(4)	-0.3373	0.1510	0.0764
C(5)	-0.2003	0.1534	0.1048
C(6)	-0.1416	0.0812	0.0943
C(7)	-0.0084	0.0781	0.1596
C(8)	0.0001	0.0731	0.3521
C(9)	-0.0316	0.1378	0.4447
C(10)	-0.1662	0.1655	0.4252
C(11)	0.0378	0.0155	0.0842
C(12)	-0.0310	0.0175	-0.0866
C(13)	0.1728	0.0094	0.0556
C(14)	-0.3852	0.1280	-0.0883
C(15)	-0.2572	0.1115	0.4872
C(16)	-0.1140	0.2754	-0.4104
C(17)	-0.1712	0.3240	-0.2675
O(18)	-0.4549	0.2587	0.0461
O(19)	-0.1291	0.0605	-0.0723
O(20)	-0.0061	0.0056	-0.2318
O(21)	-0.1841	0.2188	-0.4292
O(22)	-0.0242	0.2911	-0.4832
Br	-0.2576	0.3462	0.2386

TABLE 16

Bromodihydroisophoto -d- Santonic Lactone Acetate

Final Anisotropic Thermal Parameters ($b_{ij} \times 10^5$).

Atom	b_{11}	b_{22}	b_{33}	b_{12}	b_{23}	b_{13}
C(1)	1130	286	1820	-168	423	-2019
C(2)	1308	308	3305	68	444	-1423
C(3)	1483	450	3970	423	-704	- 271
C(4)	1001	499	3208	425	-630	- 862
C(5)	1268	397	4381	-48	191	878
C(6)	1954	452	2016	-619	243	1256
C(7)	1436	350	3689	127	146	82
C(8)	1655	461	2272	410	-822	29
C(9)	1271	398	7279	265	122	-821
C(10)	1228	412	1965	152	844	486
C(11)	1831	373	2447	-37	-62	-181
C(12)	2286	421	2613	159	479	1127
C(13)	1313	562	4398	438	1535	1489
C(14)	1908	714	4754	23	-622	-2856
C(15)	1441	422	2328	-143	-75	1805
C(16)	1308	606	2316	7	-154	-1030
C(17)	1508	642	3944	604	-562	998
O(18)	1824	604	3799	530	302	-1372
O(19)	1437	437	4157	150	258	601
O(20)	2556	668	1756	613	200	1523
O(21)	1646	300	5036	67	-70	-83
O(22)	1194	615	5869	-191	-547	-208
Br	1945	329	5460	70	161	-378
($B=6A^{02}$)	1767	584	3456	-	-	-

TABLE 17.

Bromodihydroisophoto -α- Santonic Lactone Acetate

Intramolecular Bonded Distances (Å)

C(1)-C(2)	1.48	C(7)-C(11)	1.44
C(1)-C(5)	1.57	C(8)-C(9)	1.49
C(1)-C(10)	1.47	C(9)-C(10)	1.59
C(2)-Br	1.98	C(10)-C(15)	1.53
C(2)-C(3)	1.56	C(10)-O(21)	1.56
C(3)-C(4)	1.49	C(11)-C(12)	1.55
C(3)-O(18)	1.31	C(11)-C(13)	1.51
C(4)-C(5)	1.53	C(12)-O(19)	1.37
C(4)-C(14)	1.48	C(12)-O(20)	1.26
C(5)-C(6)	1.53	C(16)-C(17)	1.60
C(6)-C(7)	1.56	C(16)-O(21)	1.34
C(6)-O(19)	1.39	C(16)-O(22)	1.19
C(7)-C(8)	1.53		

TABLE 18.

Bromodihydroisophoto -α- Santonic Lactone Acetate

Interbond Angles

C(2)C(1)C(5)	107°	C(8)C(7)C(11)	110°
C(2)C(1)C(10)	108	C(7)C(8)C(9)	115
C(5)C(1)C(10)	116	C(8)C(9)C(10)	117
C(1)C(2) Br	115	C(1)C(10)C(9)	111
C(1)C(2)C(3)	105	C(1)C(10)O(21)	109
C(3)C(2) Br	106	C(9)C(10)O(21)	106
C(2)C(3)C(4)	109	C(15)C(10)C(1)	121
C(2)C(3)O(18)	129	C(15)C(10)C(9)	111
C(4)C(3)O(18)	123	C(15)C(10)O(21)	114
C(3)C(4)C(5)	101	C(7)C(11)C(12)	100
C(3)C(4)C(14)	115	C(7)C(11)C(13)	119
C(5)C(4)C(14)	120	C(12)C(11)C(13)	111
C(1)C(5)C(4)	105	C(11)C(12)O(19)	109
C(1)C(5)C(6)	121	C(11)C(12)O(20)	133
C(4)C(5)C(6)	113	O(19)C(12)O(20)	118
C(5)C(6)C(7)	115	C(17)C(16)O(22)	122
C(5)C(6)O(19)	111	O(21)C(16)C(17)	109
C(7)C(6)O(19)	102	O(21)C(16)O(22)	129
C(6)C(7)C(8)	113	C(6)O(19)C(12)	109
C(6)C(7)C(11)	103	C(10)O(21)C(16)	123

TABLE 19.

Bromodihydroisophoto -α- Santonin Lactone Acetate

Intramolecular Non-Bonded Distances < 4Å

C(1)-C(7)	3.15	C(6)-C(9)	3.22
C(1)-C(8)	3.26	C(6)-C(10)	3.10
C(1)-C(14)	3.85	C(6)-C(13)	3.75
C(1)-C(16)	3.05	C(6)-C(14)	3.19
C(1)-O(18)	3.65	C(6)-C(15)	3.42
C(1)-O(19)	3.84	C(6)-C(20)	3.42
C(1)-O(22)	3.17	C(7)-C(10)	3.21
C(2)-C(6)	3.85	C(7)-C(15)	3.84
C(2)-C(9)	3.78	C(7)-O(20)	3.50
C(2)-C(14)	3.83	C(8)-C(12)	3.66
C(2)-C(15)	3.11	C(8)-C(15)	3.13
C(2)-C(16)	3.20	C(8)-O(19)	3.66
C(2)-O(21)	2.66	C(8)-O(21)	3.87
C(2)-O(22)	3.60	C(9)-C(11)	3.78
C(3)-C(6)	3.74	C(9)-C(16)	3.03
C(3)-C(10)	3.46	C(9)-O(22)	3.00
C(3)-C(15)	3.78	C(10)-Br	3.91
C(4)-C(7)	3.95	C(10)-C(17)	3.90
C(4)-C(10)	3.36	C(10)-O(22)	2.97
C(4)-C(15)	3.46	C(13)-O(19)	3.62
C(4)-O(19)	3.12	C(14)-O(19)	3.12
C(5)-Br	3.91	C(15)-C(16)	3.62
C(5)-C(8)	3.34	C(16)-Br	3.48
C(5)-C(9)	3.29	O(18)-Br	3.15
C(5)-C(11)	3.74	O(19)-O(20)	2.25
C(5)-C(12)	3.55	O(21)-Br	3.69
C(5)-C(15)	3.20	O(21)-O(22)	2.29
C(5)-O(18)	3.50	O(22)-Br	3.56
C(5)-O(21)	3.91		

TABLE 20.

Bromodihydroisophoto -α- Santonic Lactone Acetate

Intermolecular Distances < 4 Å

O(22) ...	$C(2)_v$	3.15	O(22) ...	$O(18)_v$	3.68
O(22) ...	$C(3)_v$	3.29	C(13) ...	Br_{iii}	3.71
C(17) ...	$O(18)_v$	3.37	C(15) ...	$O(20)_{ii}$	3.74
C(5) ...	$O(18)_{iii}$	3.41	C(16) ...	$C(2)_v$	3.76
C(16) ...	$O(18)_v$	3.44	C(9) ...	$O(20)_i$	3.78
C(15) ...	$C(12)_{ii}$	3.46	C(17) ...	$O(20)_{vi}$	3.82
C(16) ...	$C(3)_v$	3.48	O(22) ...	$O(21)_v$	3.83
O(22) ...	$C(15)_v$	3.50	C(14) ...	$O(20)_{ii}$	3.87
C(15) ...	$O(19)_{ii}$	3.57	C(6) ...	$O(18)_{iii}$	3.87
C(7) ...	$O(18)_{iii}$	3.58	C(15) ...	$O(19)_i$	3.89
C(17) ...	$C(3)_v$	3.59	O(21) ...	$C(14)_i$	3.91
O(18) ...	$C(1)_{iv}$	3.59	C(9) ...	Br_v	3.95
C(4) ...	$O(20)_{ii}$	3.62	C(15) ...	$C(6)_{ii}$	3.96
C(8) ...	$O(20)_i$	3.63	O(19) ...	$O(18)_{iii}$	3.98
C(1) ...	$C(13)_{vi}$	3.66	O(21) ...	$O(18)_v$	3.98
C(15) ...	$C(14)_i$	3.66	O(22) ...	$C(4)_v$	3.99

The subscripts refer to the following positions:

i	$x,\ y,\ z+1$	iv	$x-\frac{1}{2},\ -y+\frac{1}{2},\ -z$
ii	$-x-\frac{1}{2},\ -y,\ z+\frac{1}{2}$	v	$x+\frac{1}{2},\ -y+\frac{1}{2},\ -z+1$
iii	$x+\frac{1}{2},\ -y+\frac{1}{2},\ -z$	vi	$-x,\ y+\frac{1}{2},\ -z+\frac{1}{2}$

TABLE 21.

Bromodihydroisophoto -α- Santonic Lactone Acetate

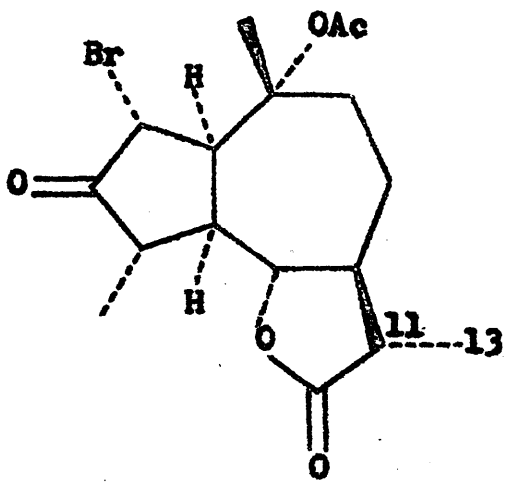
Standard Deviations of the Final Atomic Coordinates (Å)

Atom	σ(x)	σ(y)	σ(z)
C(1)	0.020	0.018	0.030
C(2)	0.021	0.021	0.033
C(3)	0.025	0.026	0.035
C(4)	0.024	0.024	0.032
C(5)	0.025	0.024	0.038
C(6)	0.027	0.024	0.031
C(7)	0.025	0.023	0.033
C(8)	0.026	0.025	0.032
C(9)	0.026	0.025	0.041
C(10)	0.024	0.023	0.032
C(11)	0.027	0.024	0.032
C(12)	0.028	0.025	0.034
C(13)	0.026	0.026	0.036
C(14)	0.031	0.032	0.040
C(15)	0.026	0.022	0.030
C(16)	0.023	0.028	0.031
C(17)	0.026	0.028	0.040
O(18)	0.019	0.018	0.024
O(19)	0.017	0.017	0.023
O(20)	0.020	0.018	0.022
O(21)	0.018	0.015	0.023
O(22)	0.017	0.019	0.027
Br	0.003	0.002	0.005

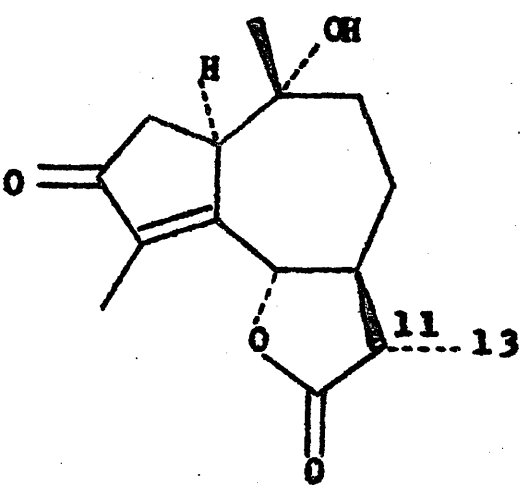
in Table 20. Standard deviations of the positional parameters were estimated from the least squares residuals in the usual way, and are listed in Table 21. The average standard deviation of a C-C bond is 0.04 Å, that of a C-O bond 0.03 Å, and of a C-Br bond 0.02 Å. The average standard deviation in a bond angle is 2°.

3. (8) DISCUSSION

The analysis has established the structure and relative stereochemistry of bromodihydroisophoto-α-santonic lactone acetate as (III); it follows therefore, that the stereochemistry of isophoto-α-santonic lactone is as in (IV), the absolute stereochemistry shown being firmly established chemically.



(III)



(IV)

As predicted by Barton, O(19) in the lactone ring is α and C(11)β. The methyl group in the lactone ring, however, has the opposite configuration (α) to that expected (β) and this casts doubt on the generally accepted configuration (β) of the corresponding methyl group in (-)-α-santonin itself. Full discussion on this subject appears in Chapter 4 of this thesis.

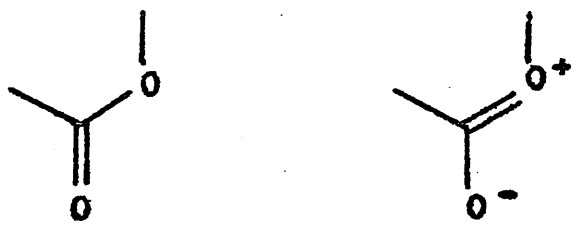
The stereochemistry of the ring junction of the five and seven-membered ring is clearly defined. The rings are cis fused through equatorial bonds. The methyl group, C(15), has the β and the C(10)-acetoxy substituent the α configuration.

De Mayo and Reid (1961) have suggested a mechanism for the transformation, but as yet there is no evidence for their postulated intermediate.

In bromodihydroisophoto-α-santonic lactone acetate, the average carbon-carbon single-bond length is 1.523 Å which appears to be rather short, but within the limits of experimental error cannot be said to differ significantly from the standard value of 1.545 Å in diamond.

The carbon-oxygen single bonds fall into two categories: those adjacent to a carbon oxygen double bond have a mean value of 1.35 Å whereas the others have an average value of 1.47 Å.

Calculated distances for the carbon-oxygen bond can be obtained from the method due to Pauling (1960) from covalent bond radii and electronegativity differences in bonding atoms. The carbon oxygen double and single bond lengths are theoretically predicted as 1.21 Å and 1.43 Å respectively. In the completely ionised carboxylate group the character and length of the carbon oxygen bond is midway between these two extremes, and so the difference in carbon oxygen bond lengths found in the present structure would seem to indicate that in lactone and ester groupings, as in carboxylic acids, the resonance structure shown in Fig. 18 (b) makes a significant contribution.



(a) (b)

Fig. 18 Resonance forms present in the ester and lactone groups

This effect is closely paralleled in other lactones and esters, e.g. α-bromopicrotoxinin (Craven, 1962), clerodin bromolactone (Paul, Sim, Hamer and Robertson, 1962) and bromogeigerin acetate (Hamilton, McPhail and Sim, 1962) have

values of 1.37 Å, 1.34 Å, 1.29 Å and 1.47 Å, 1.47 Å, 1.46 Å for the short and long carbon-oxygen bonds respectively. Furthermore the angles about C(12) and C(16) conform to a pattern observed in carboxylic acids (Ahmed and Cruickshank, 1953; Ferguson and Sim, 1961); the angles C(11) C(12) O(19) and C(17) C(16) O(21) are $109\frac{1}{2}^{\circ}$ and 109° respectively, approximately tetrahedral, whereas the average of the angles C(11) C(12) O(20), O(19) C(12) O(20), C(17) C(16) O(22) and O(21) C(16) O(22) exceeds 120°. The effect may be due to repulsion by the lone pairs of electrons on the atoms O(20) and O(22).

The average carbon-oxygen double bond length, 1.25 Å, is not significantly longer than the theoretically predicted value of 1.21 Å (Pauling 1960). The carbon-bromine bond length of 1.98 Å appears to be slightly longer than the average value of 1.94 Å reported for various alkyl bromides (Sutton et al., 1958), but is comparable with the values of 1.98 Å reported in α-bromopicrotoxinin (Craven, 1962) and 1.99 Å in bromogeigerin acetate (Hamilton, McPhail and Sim, 1962).

The cycloheptane ring adopts a chair conformation but is somewhat flattened, for the valency angles are consistently greater than tetrahedral, the mean value being $115\frac{1}{2}^{\circ}$. Similar large values for the valency angles in seven

and higher membered rings have been noted: 116.5° in isoclovene hydrochloride (Clunie and Robertson, 1961), 116.5° in cyclononylamine hydrobromide (Bryan and Dunitz, 1960).

In the five-membered rings the average valency angle is 105°. Average valency angles consistently smaller than tetrahedral have been reported, inter alia, for the five membered rings in hydroxy-L-proline, 106°, (Donohue and Trueblood, 1952), isoclovene hydrochloride, 105°, (Clunie and Robertson, 1961) and in the five-membered ring which is not adjacent to the benzene ring in echitamine bromide methanol solvate, 106° (Hamilton, Hamor, Robertson and Sim, 1962). These values are consistent with non-planarity and consequent angle deformations in cyclopentane (Pitzer and Donath, 1959).

The mean plane through the atoms C(1), C(2), C(3) and C(5) of the five-membered carbocyclic ring was calculated by the method of Shomaker et al. (1959). The equation of the plane is $0.429X + 0.686Y - 0.587Z - 0.579 = 0$, and the deviations from the plane are C(1) - 0.012, C(2) + 0.012, C(3) - 0.007 and C(5) + 0.008. The displacement of 0.541 Å of C(4) is highly significant and establishes the presence of the envelope conformation as defined by Brutcher, Roberts, Barr and Pearson (1959).

In himbacine hydrobromide, Fridrichsons and Mathieson (1962) have reported that the γ-lactone system (b), (c), (d) and (e) (Fig. 19),

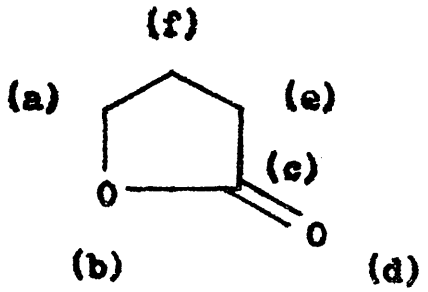


Fig. 19. γ-lactone system

corresponding to C(11), C(12), O(19) and O(20) in bromodihydroisophoto-α-santonic lactone acetate, is planar and nearly coplanar with (a) while (f) lies markedly out of the plane of the ring. McPhail (1963) considers that the partial double bond character of the (b) (c) bond should lead to planarity of atoms (a), (b), (c), (d) and (e) and has established the validity of his proposal in bromogeigerin acetate and also in the lactone ring of himbacine hydrobromide by applying the χ^2 test (Fisher and Yates, 1957). For bromogeigerin acetate $\chi^2 = 2.1$ and for himbacine hydrobromide $\chi^2 = 4.2$: these values mean that there is no significant deviation of (a), (b), (c), (d) or (e) from the planes.

For bromodihydroisophoto-α-santonic lactone acetate, the equation of the mean plane through (a), (b), (c), (d) and (e) is $0.530X + 0.816Y - 0.230Z - 0.292 = 0$. The

displacement, 0.60 Å, of (f), C(7) is significant and demonstrates the envelope conformation of the ring. χ^2 = 5.1 and this shows that bromodihydroisophoto-α-santonic lactone acetate conforms to the pattern predicted by McPhail. The results of the mean plane calculations through the atoms of the γ-lactone rings discussed, and also the γ-lactone ring in 2-Br-α-santonin, are compiled in Table 22.

The distance between C(6) and C(15) in a model with standard bond lengths and angles is 2.1 Å, shorter than that found, 3.41 Å, in the crystal, and indicates considerable forces pushing these atoms apart. The five membered carbocyclic ring incurs considerable strain in adopting the envelope conformation where C(4) is out of the plane of the ring.

Of the intermolecular distances (Table 20), the C....O contacts are the shortest, the lowest values being 3.15 Å, 3.28 Å and 3.37 Å. C....O contacts of comparable lengths were quoted for several alkaloids in Chapter 2. (8), and values of 2.96 Å and 3.07 Å have been reported in bromogeigerin acetate (Hamilton, McPhail and Sim, 1962). The shortest C....C contact is 3.46 Å; none of the contacts appears to be abnormal.

The crystal packing diagram projected down (001) demonstrates clearly the stereochemistry of the methyl group on the lactone ring (Fig. 17).

TABLE 22.

Mean Plane Calculations for some γ-Lactone Rings (see Fig. XIX).

	Atoms omitted	$\Sigma\omega\Delta^2 10^{-4}$	χ^2	P%
Bromodihydroisophoto -d- santonic lactone acetate	None	1851	272	<0.1
	C(a)	1234	181	<0.1
$\sigma^2 = 6.8 \times 10^{-4}$	O(b)	1804	265	<0.1
	C(c)	1789	263	<0.1
	O(d)	1510	222	<0.1
	C(e)	720	106	<0.1
	C(f)	35	5.1	>5
Bromogeigerin acetate	None	1903	476	<0.1
$\sigma^2 = 4 \times 10^{-4}$	C(a)	1442	356	<0.1
	O(b)	1872	468	<0.1
	C(c)	1880	470	<0.1
	O(d)	1496	370	<0.1
	C(e)	445	111	<0.1
	C(f)	8.5	2.1	95>P>5
Himbacine hydrobromide	None	1794	224	<0.1
	C(a)	1362	158	<0.1
$\sigma^2 = 8 \times 10^{-4}$	O(b)	1776	222	<0.1
	C(c)	1790	224	<0.1
	O(d)	1510	189	<0.1
	C(e)	283	35	<0.1
	C(f)	33.7	4.2	>5
2-Br -d-santonin	None	1804	188	<0.1
	C(a)	1255	131	<0.1
$\sigma^2 = 9.6 \times 10^{-4}$	O(b)	1787	186	<0.1
	C(c)	1722	179	<0.1
	O(d)	1542	161	<0.1
	C(2)	523	54	<0.1
	C(f)	0.8	0.01	>99

Displacements from the Best Plane -C(f) omitted- in Å.

	Bromodihydroisophoto -α- santonic lactone acetate	*Bromoeigerin acetate*
C(a)	-0.018	-0.010
O(b)	0.033	0.005
C(c)	-0.041	0.022
O(d)	0.005	-0.015
C(e)	0.020	-0.002
C(f)	0.595	-0.630

	Himbacine hydrobromide	*2-Br -α- santonin*
C(a)	-0.019	-0.004
O(b)	-0.035	0.003
C(c)	-0.038	0.006
O(d)	0.002	-0.005
C(e)	+0.020	-0.001
C(f)	-0.612	0.614

PART IV.

THE STRUCTURE OF SANTONIN ; X-RAY ANALYSIS OF 2 - BROMO -α- SANTONIN

4. (1) INTRODUCTION

Santonin ($C_{15}H_{18}O_3$) is a colourless, crystalline lactone, obtained from the unexpanded flowerheads of Artemesia cina and other species of Artemesia growing in Cashmere, Mexico, Turkestan and Western Tibet. It is a valuable anthelmintic and is cultivated for medicinal purposes particularly in Turkestan.

Santonin is one of the eudesmane group of sesquiterpenes and classical chemical methods have been employed to establish its structure (Clemo, Haworth and Walton, 1929; 1930 . Clemo and Haworth, 1930) and stereochemistry (see Cocker and McMurray, 1960, for a summary).

Examination of the literature shows that two viewpoints have been presented concerning the configuration of the methyl group at C(11) in naturally occurring santonins. Miki (1955) assumed the quasi-equatorial orientation of the methyl group at C(11) in (-)-α-santonin because complete stereo-specificity was observed in decarboxylation of 11-carboxysantonin and its stereoisomers. Conformation of this assignation was made by Abe, Miki, Sumi and Toga (1956) who converted (-)-α-santonin to 11-noreusantonan-11-ol and compared its molecular rotation to that of atrolactic acid.

Corey (1955) assigned the β-position to the methyl group at C(11) in (-)-α-santonin on the basis of the fact that

β-desmotroposantonins are more stable than the α-isomers in an acidic medium, while Woodward and Yates (1954) and Cocker and McMurray (1955) have reached the same conclusion.

It is clear that doubt exists about the stereochemistry at C(11) and one consequence of this is that the possibility of epimerisation at C(11) during the phototransformation to isophotosantonic lactone cannot be ruled out.

Transparent needle shaped crystals of 2-bromo-α-santonin supplied by Professor Barton were used in the x-ray structure analysis. Preliminary work - crystal data and Patterson projections - was carried out by A. C. MacDonald (1962) during B.Sc. thesis work.

At an early stage in the analysis, Nakasaki and Arawaka (1962) published work supporting the choice of configuration made by Miki (1955), but the x-ray analysis continued in an attempt to furnish independent evidence.

4. (2) EXPERIMENTAL

Rotation, oscillation and precession photographs were taken about the needle (c) axis for unit cell data. The density of the crystal was found by a flotation technique in aqueous potassium iodide solution, and agreed well with the calculated density.

Intensity data for the hk0.....hk6 layer lines were collected with a Weissenberg equi-inclination camera using the multiple-film technique of Robertson (1934) and estimated visually employing a calibrated step wedge. Film factors were determined by the method of Rossman (1956), and the intensities corrected for Lorentz, polarization, and rotation factors for upper layers. The resulting structure amplitudes were scaled by comparison with a precession photograph of the 0kl zone, and later placed on an absolute scale by reference to the calculated structure amplitudes |Fc| . In all 1434 independent intensities were estimated, and of these, 157 were smaller than the least observable value.

The space group was uniquely determined from systematic halvings in the x-ray spectra. A small crystal was used for the intensity survey and no absorption corrections were made.

4. (3) CRYSTAL DATA

$C_{15}H_{17}O_3Br$ M = 325.2

Orthorhombic

a = 7.34 Å ± 0.01 Å

b = 23.34 Å ± 0.02 Å

c = 8.28 Å ± 0.03 Å

Volume of the unit cell = 1418 $Å^3$

For Z = 4, ρ(calculated) = 1.522 g./ml.

ρ(measured) = 1.525 g./ml.

ρ(measured)100) = 664

Absorption coefficient for x-rays (λ=1.542) = 43.7 cm.$^{-1}$

Systematic absences; (h00) = 2n + 1

(0k0) = 2n + 1

(00ℓ) = 2n + 1

Space group $P2_12_12_1$ - D_2^4.

4. (4) LOCATION OF THE HEAVY ATOM

The determination of the heavy atom position in 2-bromo-α-santonin paralleled closely that in bromodihydroisophoto-α-santonic lactone acetate as the space group is the same and there is only one heavy atom in each of the equivalent positions.

The two-dimensional Patterson projections, P(VW) and P(UV), were computed using 215 and 200 terms respectively and are shown in Figs. 20 and 21. Peaks A, B and C in Fig.20 correspond to the vectors $2y_{Br}$, $\frac{1}{2}$; $\frac{1}{2}$, $\frac{1}{2}-2z_{Br}$ and $\frac{1}{2}-2y_{Br}$, $2z_{Br}$ respectively.

The projection down (001) in Fig. 21 could be interpreted in two ways. Peaks D, E, F and D, M, N are both concordant with the vectors $2x_{Br}$, $\frac{1}{2}$; $\frac{1}{2}$, $\frac{1}{2}-2y_{Br}$ and $\frac{1}{2}-2x_{Br}$, $2y_{Br}$, but the former set gave the same value of y_{Br} as the projection down (100) whereas the latter set yielded a different value. Solution of the Harker sections which were subsequently computed accorded with the solution based on the vectors at D, E and F. Closer examination of

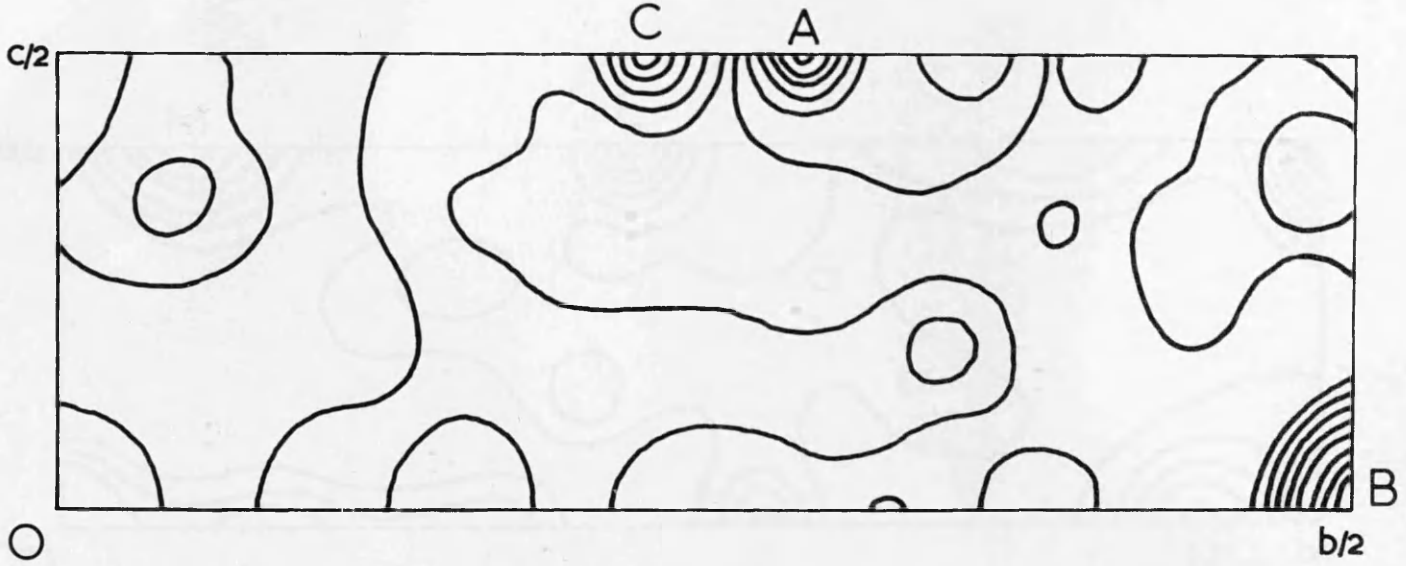


Fig. 20. 2-Bromo-α-santonin.

(100) Patterson projection. Contours are at equal and arbitrary intervals. Br-Br vectors discussed in the text are marked A, B and C.

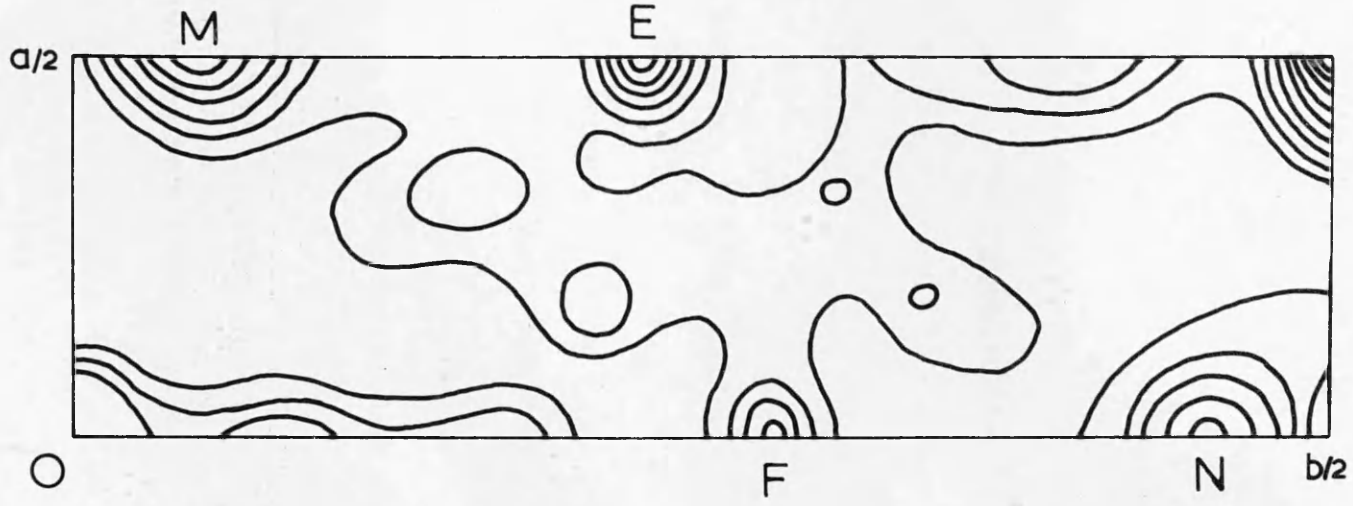


Fig. 21. 2-Bromo-2-santonin.

(OOl) Patterson projection. Contours are at equal and arbitrary intervals. Br-Br vectors discussed in the text are marked, D, E, F, M and N.

the peaks at M and N showed that they were more diffuse than those at E and F, and could possibly be accounted for by the presence of a planar ring at $x = \frac{1}{4}$ and perpendicular to (001). The solution of the Patterson projections indicated that both the x and z coordinates of the heavy atom are close to screw axes.

The three Harker sections at $(\frac{1}{2}VW)$, $(U\frac{1}{2}W)$ and $(UV\frac{1}{2})$ were computed using all the three-dimensional data. The section at $(\frac{1}{2}VW)$ contained the normally expected vector at $\frac{1}{2}$, $\frac{1}{2}-2y_{Br}$, $2z_{Br}$ and a further vector at $2x_{Br}$, $\frac{1}{2}$, $\frac{1}{2}-2z_{Br}$ arising from the value $x_{Br} = \sim\frac{1}{4}$. These are marked P and Q respectively in Fig. 22. The corresponding vectors at $\frac{1}{2}-2x_{Br}$, $2y_{Br}$, $\frac{1}{2}$ and $\frac{1}{2}$. $\frac{1}{2}-2y_{Br}$, $2z_{Br}$ in the section at $(UV\frac{1}{2})$ are marked R and S respectively in Fig. 23. The Harker section at $(U\frac{1}{2}W)$ contained the vector $2x_{Br}$, $\frac{1}{2}$, $\frac{1}{2}-2z_{Br}$ marked T in Fig. 24.

Graphs were drawn to determine as accurately as possible the positions of the peak maxima. These established that the x coordinate of the bromine atom was closer to the value of $\frac{1}{4}$ than the z coordinate.

The vectors were consistent with the fundamental set

$$x/a = 0.233 \qquad y/b = 0.139 \qquad z/c = 0.230$$

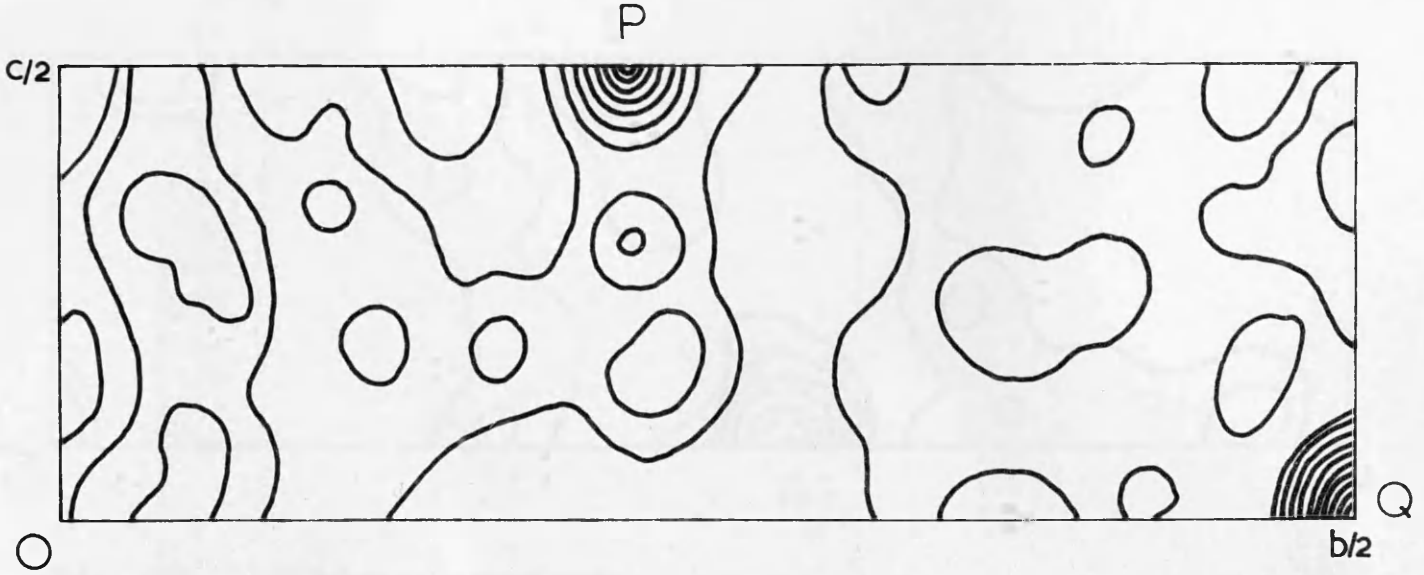


Fig. 22. 2-Bromo-α-santonin.

Harker section P(½VW) of the 3D Patterson synthesis.
Contours are at equal and arbitrary intervals.
Br-Br vectors discussed in the text are marked P and Q.

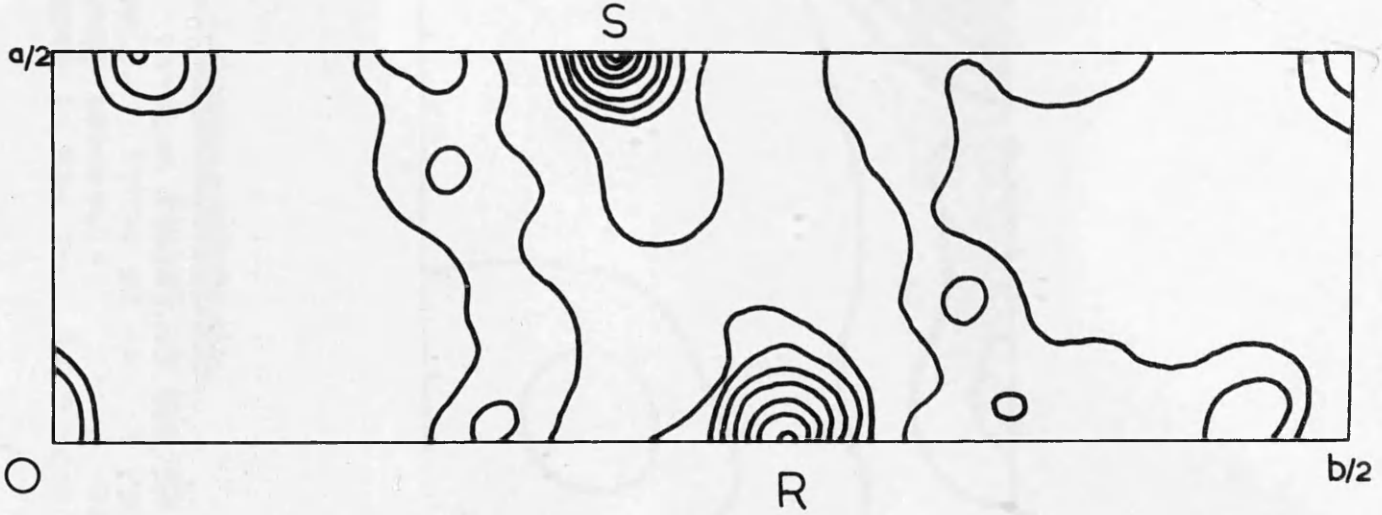


Fig. 23. 2-Bromo-2-santonin.

Harker section P(UV½) of the 3D Patterson synthesis.
Contours are at equal and arbitrary intervals.
Br-Br vectors discussed in the text are marked R and S.

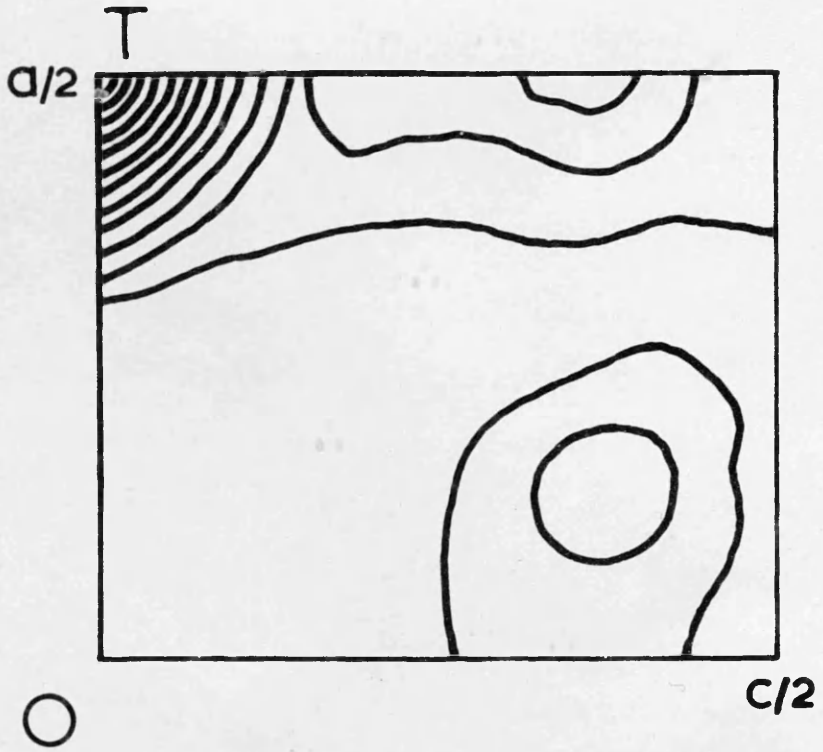


Fig. 24. 2-Bromo-α-santonin.

Harker section P(U½W) of the 3D Patterson synthesis. Contours are at equal and arbitrary intervals. The Br-Br vector discussed in the text is marked 2.

4. (5) SOLUTION OF THE STRUCTURE

The proximity of x_{Br} and z_{Br} to screw axes introduces centres of symmetry into any Fourier map whose phases are based on the bromine atom alone. The spurious symmetry will be attenuated if the bromine is moved to a more general position, and thus small adjustments were made to the coordinates and structure factors calculated for each resultant heavy atom position. The lowest discrepancy, 43.6%, arose from the coordinates x/a = 0.233, y/b = 0.139 and z/c = 0.217.

Combining the phases from the calculated structure factors with the observed structure amplitudes, a three-dimensional Fourier synthesis was calculated. In spite of spurious mirror planes, the position and orientation of the molecule in the unit cell was defined by the general trend of electron density, and the appearance in an unequivocable position on a mirror plane of the carbon atom bonded to the bromine atom provided confirmation. The cyclohexadienone ring system lay in the bc plane and along with its methyl and carbonyl constituents was disentangled from its ghosts. This ring was responsible for the appearance of peaks M and N in the Patterson projection down (001) (Fig. 21). Knowledge of the structure and stereochemistry of the molecule allowed all the atoms to be located with the exception of the

controversial methyl group on the lactone ring. Realisation of the fact that the molecule as it appeared in the Fourier map was the mirror image of that which appears in the literature was important in solving the structure.

Coordinates were assigned to the atoms, each of which was allotted its correct chemical type, and they were included in the calculation of a second set of structure factors. The discrepancy fell to 39.1% and the improved phases were employed in the computation of a second three-dimensional electron-density map. A peak of just under $2e/\mathring{A}^3$ which may have represented the disputed methyl group was evident. Atomic coordinates were adjusted and a third cycle of structure factors (R = 34.5%) and Fourier synthesis calculated. C(13) was now prominent at $>3e/\mathring{A}^3$, and with the inclusion of all the atoms of the structure the value of R fell to 30.4% in the fourth round of structure factor calculation, and fell to 29.1% in the fifth.

The progress of the analysis is summarised in Table 23.

4. (6) REFINEMENT

The phases from the fifth set of calculated structure factors were employed in computing both F_o and F_c Fourier maps and back-shift corrections for errors due to termination of series applied. Individual isotropic temperature factors

TABLE 23.

2 - Brom -d- Santonin

Course of the Structure Determination

Operation	Atoms included	R(%)
2D Patterson syntheses		
3D Harker Syntheses		
1st 3D Fourier syntheats	Br	43.6
2nd " " "	Br + 14(C) + 3(O)	39.1
3rd " " "	Br + 14(C) + 3(O)	34.5
4th " " "	Br + 15(C) + 3(O)	30.4

TABLE 24.

2 - Bromo -d- Santonin

Course of Refinement

Operation	Atoms included	R(%)	$\Sigma\omega\Delta^2$
5th 3D Fourier synthesis (F_o and F_c)	Br + 15(C) + 3(O)	29.1	-
6th " " " "	Br + 15(C) + 3(O)	25.4	-
7th " " " "	Br + 15(C) + 3(O)	24.5	-
8th " " " "	Br + 15(C) + 3(O)	23.7	-
1st Least squares cycle	Br + 15(C) + 3(O)	23.3	295
2nd " "	Br + 15(C) + 3(O)	20.0	197
3rd " "	Br + 15(C) + 3(O)	18.2	164
4th " "	Br + 15(C) + 3(O)	17.5	507
5th " "	Br + 15(C) + 3(O)	15.2	399
9th 3D Fourier synthesis (F_o)	Br + 15(C) + 3(O)	15.2	

were assigned. Four such rounds of calculations reduced the value of R to 23.7%.

The next stage of refinement consisted of adjustment of positional and anisotropic thermal parameters by means of Dr. J. S. Rollett's least-squares programme (1961). Half-scale scattering factors and half-shift corrections were employed. Five cycles were carried out and the final discrepancy, with unobserved terms excluded, was 15.2%.

The course of refinement is set down in Table 24. The rise of $\sigma\omega\Delta^2$ in cycle IV was associated with an increase from 10 to 16 in the value of F^* in the weighting system which was identical with that used in the refinement of hunterburnine β-methiodide (Chapter 2. (6)). The rather large drop in R in cycle V was a consequence of the re-estimation of some forty intensities.

On the basis of the final phase constants a final three-dimensional Fourier was computed.

4. (7) RESULTS

The final positional parameters are recorded in Table 25 and the standard deviations, estimated from the least-squares residuals in the usual manner, are shown in Table 26. The average estimated standard deviation of a C-C bond is about 0.05 Å, of a C-O bond, about 0.04 Å and

TABLE 25.

2 - Bromo -α- Santonin

Final Atomic Coordinates

Atom	x/a	y/b	z/c
C(1)	0.2283	0.2590	0.1818
C(2)	0.2292	0.2188	0.2920
C(3)	0.2309	0.2332	0.4720
C(4)	0.2326	0.2944	0.5048
C(5)	0.2391	0.3349	0.3926
C(6)	0.2668	0.3980	0.4132
C(7)	0.4452	0.4146	0.3400
C(8)	0.4485	0.4067	0.1568
C(9)	0.4087	0.3444	0.1238
C(10)	0.2322	0.3191	0.2096
C(11)	0.4672	0.4730	0.4175
C(12)	0.3948	0.4633	0.5779
C(13)	0.6763	0.4966	0.4267
C(14)	0.2214	0.3045	0.6881
C(15)	0.0552	0.3468	0.1346
O(16)	0.2234	0.1986	0.5682
O(17)	0.2712	0.4196	0.5775
O(18)	0.4189	0.4876	0.7097
Br	0.2255	0.1381	0.2453

TABLE 26.

2 - Bromo -α- Santonin

Standard Deviations of the Final Atomic Coordinates (Å)

Atom	σ(x)	σ(y)	σ(z)
C(1)	0.034	0.030	0.031
C(2)	0.029	0.021	0.028
C(3)	0.031	0.025	0.033
C(4)	0.030	0.025	0.030
C(5)	0.031	0.028	0.030
C(6)	0.032	0.024	0.032
C(7)	0.037	0.029	0.035
C(8)	0.038	0.032	0.034
C(9)	0.033	0.029	0.039
C(10)	0.029	0.025	0.030
C(11)	0.035	0.030	0.043
C(12)	0.032	0.026	0.038
C(13)	0.043	0.041	0.049
C(14)	0.038	0.030	0.029
C(15)	0.033	0.029	0.036
O(16)	0.028	0.019	0.023
O(17)	0.024	0.020	0.021
O(18)	0.023	0.022	0.026
Br	0.003	0.003	0.002

TABLE 27.

2 - Bromo -α- Santonin

Intramolecular Bonded Distances (Å)

C(1) - C(2)	1.31	C(6) - O(17)	1.45
C(1) - C(10)	1.42	C(7) - C(8)	1.53
C(2) - Br	1.92	C(7) - C(11)	1.51
C(2) - C(3)	1.53	C(8) - C(9)	1.51
C(3) - C(4)	1.45	C(9) - C(10)	1.59
C(3) - O(16)	1.14	C(10) - C(15)	1.58
C(4) - C(5)	1.32	C(11) - C(12)	1.45
C(4) - C(14)	1.54	C(11) - C(13)	1.63
C(5) - C(6)	1.50	C(12) - O(17)	1.34
C(5) - C(10)	1.56	C(12) - O(18)	1.24
C(6) - C(7)	1.49		

TABLE 28.

2 - Bromo -α- Santonin

Intramolecular Non-bonded Distances <4 Å.

C(1) - C(4)	2.80	C(6) - C(13)	3.79
C(1) - C(6)	3.78	C(6) - C(14)	3.17
C(1) - C(8)	3.81	C(6) - C(15)	3.03
C(1) - O(16)	3.50	C(6) - O(18)	3.41
C(2) - C(5)	2.84	C(7) - C(10)	2.93
C(2) - C(9)	3.50	C(7) - C(15)	3.69
C(2) - C(14)	3.84	C(7) - O(18)	3.51
C(2) - C(15)	3.50	C(8) - C(12)	3.75
C(3) - C(6)	3.89	C(8) - C(13)	3.49
C(3) - C(10)	2.95	C(8) - C(15)	3.21
C(4) - C(7)	3.49	C(8) - O(17)	3.72
C(4) - C(9)	3.60	C(9) - C(11)	3.89
C(4) - C(15)	3.54	C(10) - C(14)	3.98
C(4) - O(17)	3.00	C(10) - O(17)	3.86
C(5) - C(8)	3.00	C(13) - O(17)	3.66
C(5) - C(11)	3.64	C(13) - O(18)	3.02
C(5) - C(12)	3.56	C(14) - O(17)	2.87
C(5) - O(16)	3.50	O(16) - Br	3.02
C(6) - C(9)	2.90		

TABLE 29.

2 - Bromo -α- Santonin

Intermolecular Distances < 4Å.

C(7)	...	$O(16)_{vi}$	3.42	C(3)	...	$C(4)_{vi}$	3.74
O(18)	...	$C(6)_{iii}$	3.44	C(14)	...	$C(2)_{vi}$	3.77
O(18)	...	$C(11)_{iii}$	3.44	C(3)	...	$C(3)_{vi}$	3.78
C(13)	...	$O(18)_{viii}$	3.49	O(16)	...	$C(4)_{vi}$	3.79
C(9)	...	$O(16)_{vi}$	3.58	O(17)	...	Br_{vi}	3.85
O(16)	...	$C(15)_{vi}$	3.62	Br	...	$C(9)_{v}$	3.86
C(5)	...	$O(16)_{vi}$	3.65	O(16)	...	$C(5)_{vi}$	3.88
C(4)	...	$O(16)_{vi}$	3.66	C(8)	...	$O(16)_{vi}$	3.91
C(2)	...	$C(14)_{vi}$	3.66	Br	...	$C(14)_{vi}$	3.92
C(13)	...	Br_{vii}	3.67	O(18)	...	$C(15)_{iii}$	3.92
O(18)	...	$C(7)_{iii}$	3.68	C(3)	...	$C(14)_{vi}$	3.94
O(18)	...	$C(8)_{iii}$	3.68	C(3)	...	$O(16)_{vi}$	3.96
C(12)	...	Br_{vi}	3.69	C(14)	...	$C(9)_{i}$	3.97
O(18)	...	Br_{vi}	3.72	C(14)	...	Br_{vi}	3.97
C(4)	...	$C(3)_{vi}$	3.72	C(15)	...	Br_{v}	3.98
				O(18)	...	$C(12)_{iii}$	3.99

The subscripts refer to the following positions:

i	$x,$	$y,$	$z + 1$	v	$x - \frac{1}{2},$	$-y + \frac{1}{2},$	$-z$
ii	$x,$	$y,$	$z - 1$	vi	$x + \frac{1}{2},$	$-y + \frac{1}{2},$	$-z + 1$
iii	$-x + \frac{1}{2},$	$-y + 1,$	$z + \frac{1}{2}$	vii	$-x + 1,$	$y + \frac{1}{2},$	$-z + \frac{1}{2}$
iv	$x + \frac{1}{2},$	$-y + \frac{1}{2},$	$-z$	viii	$-x + 1\frac{1}{2},$	$-y + 1,$	$z - \frac{1}{2}$

TABLE 30.

2 - Bromo -α- Santonin

Interbond Angles

C(2) C(1) C(10)	126°	C(6) C(7) C(11)	99°
C(1) C(2) Br	124	C(8) C(7) C(11)	122
C(3) C(2) Br	114	C(7) C(8) C(9)	107
C(1) C(2) C(3)	122	C(8) C(9) C(10)	116
C(2) C(3) C(4)	113	C(1) C(10) C(5)	113
C(2) C(3) O(16)	122	C(1) C(10) C(9)	108
C(4) C(3) O(16)	125	C(1) C(10) C(15)	109
C(3) C(4) C(5)	125	C(5) C(10) C(9)	109
C(3) C(4) C(14)	109	C(5) C(10) C(15)	108
C(5) C(4) C(14)	126	C(9) C(10) C(15)	110
C(4) C(5) C(6)	129	C(7) C(11) C(12)	102
C(4) C(5) C(10)	121	C(7) C(11) C(13)	115
C(6) C(5) C(10)	110	C(12) C(11) C(13)	111
C(5) C(6) C(7)	109	C(11) C(12) O(17)	105
C(5) C(6) O(17)	117	C(11) C(12) O(18)	133
C(7) C(6) O(17)	104	O(17) C(12) O(18)	116
C(6) C(7) C(8)	113	C(6) O(17) C(12)	108

TABLE 31.

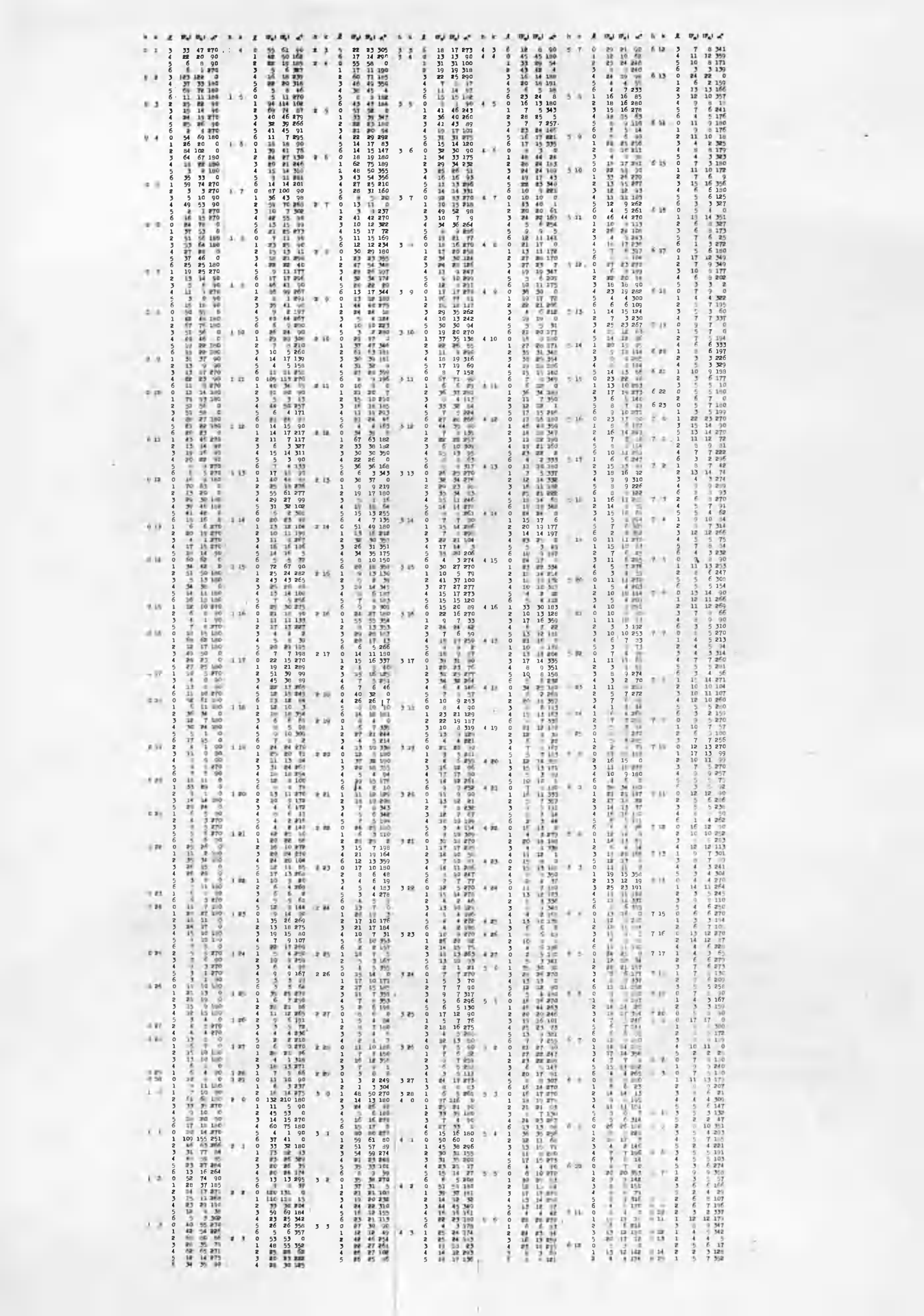
2 - Bromo - α - Santonin

Final Anisotropic Thermal Parameters ($b_{ij} \times 10^5$)

Atom	b_{11}	b_{22}	b_{33}	b_{12}	b_{23}	b_{13}
C(1)	4001	298	2694	-162	181	157
C(2)	2845	164	2323	-368	-238	615
C(3)	1805	240	3239	-75	-169	1165
C(4)	1932	240	2246	-240	-294	418
C(5)	1640	346	1911	105	-128	-1272
C(6)	2122	224	2306	30	-196	-707
C(7)	3567	201	2356	-123	177	-536
C(8)	4234	358	958	-76	-10	287
C(9)	3197	245	2989	92	-132	912
C(10)	2375	280	2492	-367	462	-508
C(11)	2873	220	3682	-45	69	465
C(12)	2624	155	2960	394	-732	-794
C(13)	4636	539	4527	-905	-205	452
C(14)	4642	310	1814	-98	179	-613
C(15)	2963	275	2273	-45	-202	-927
O(16)	5884	270	2951	-605	218	-601
O(17)	3119	358	2173	-32	-478	530
O(18)	3476	380	5819	-26	-865	583
Br	3629	244	3173	-172	-154	267
($B=3A^{02}$)	2752	264	2099	-	-	-

Table 32. 2-Bromo-α-santonin.

Final measured and calculated values of the structure factors. Unobserved terms have been omitted.



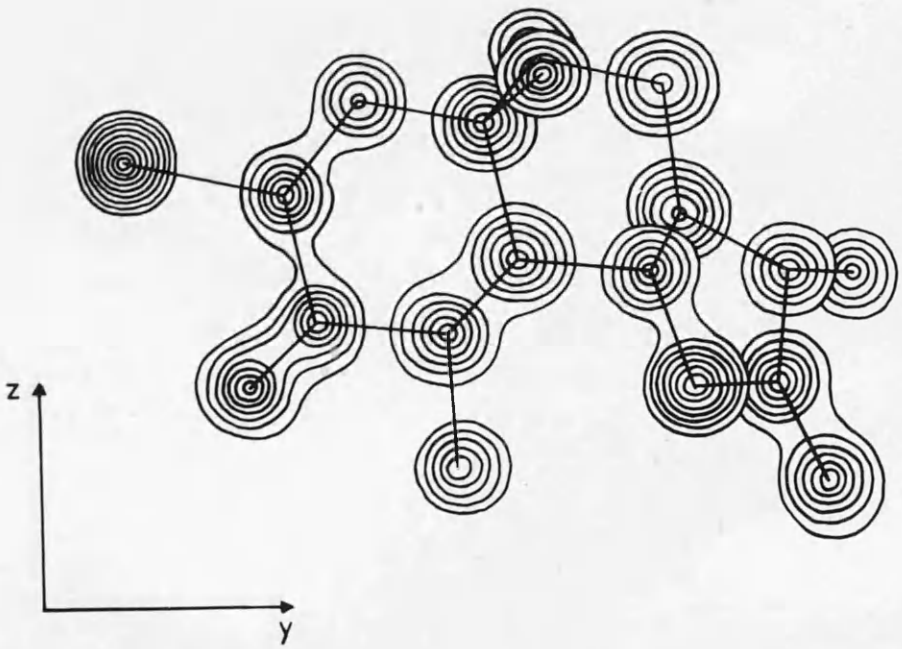


Fig. 25. 2-Bromo-α-santonin.

Superimposed contoured sections of the final 3D Fourier synthesis covering the region of one molecule. The sections are drawn parallel to (100). Contours are drawn at intervals of 1 electron/Å^3 with the exception of the bromine atom for which the intervals are at 4 electrons/Å^3.

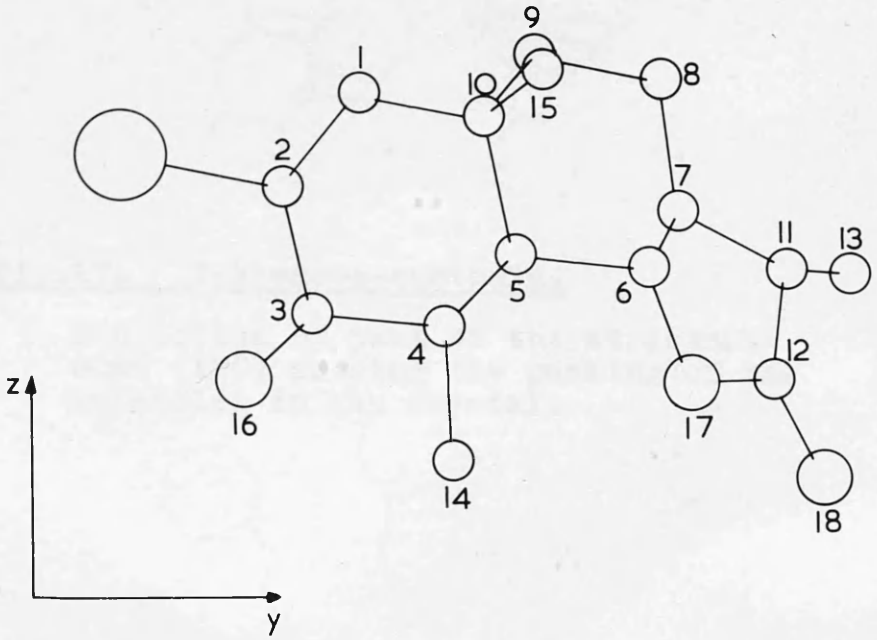
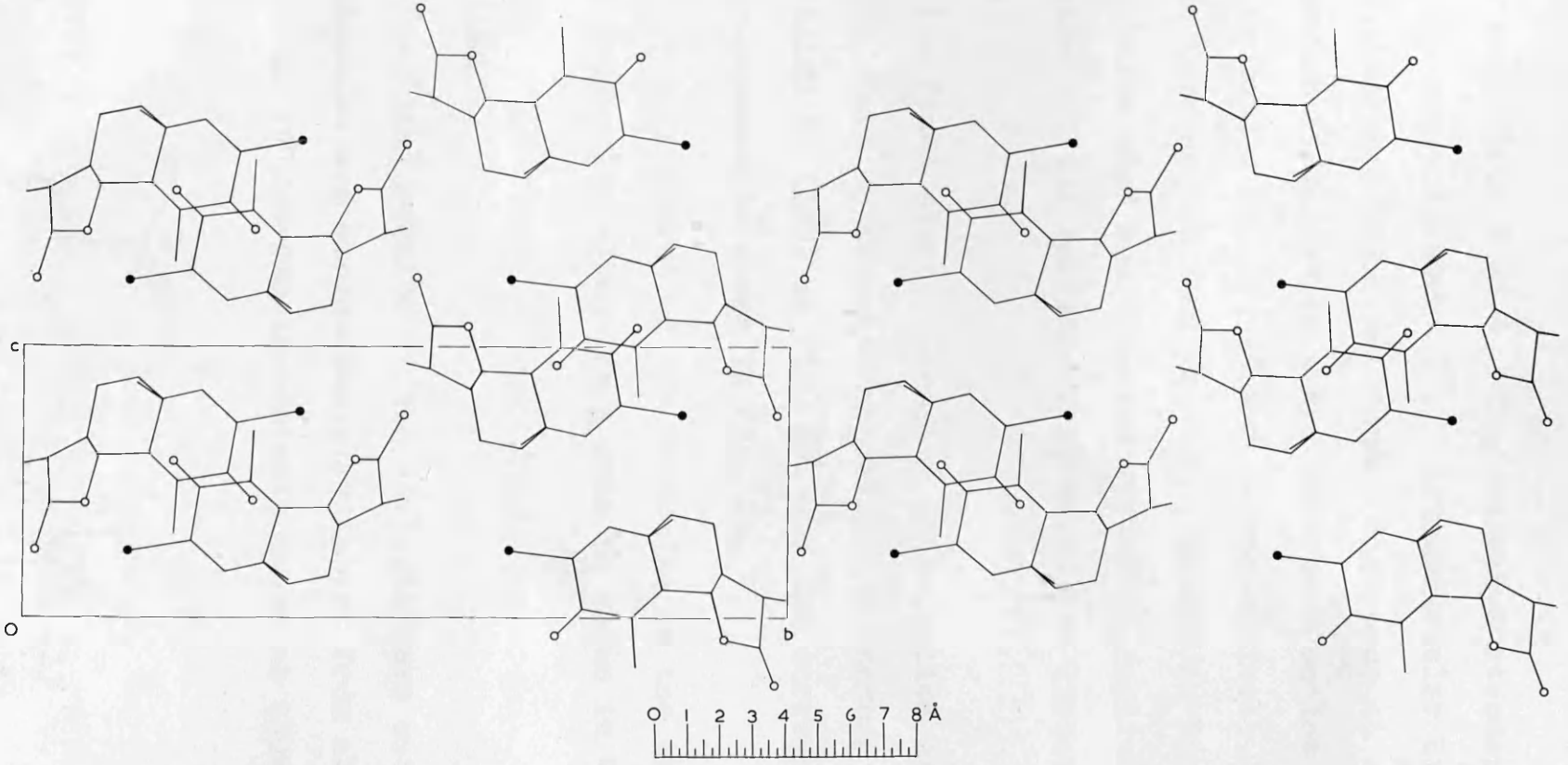


Fig. 26. 2-Brom-α-santonin.
Atomic arrangement corresponding to Fig. 25.

Fig.27. 2-Bromo-α-santonin.

Projection of part of the structure down (100) showing the packing of the molecules in the crystal.


c
O
b
0 1 2 3 4 5 6 7 8 Å

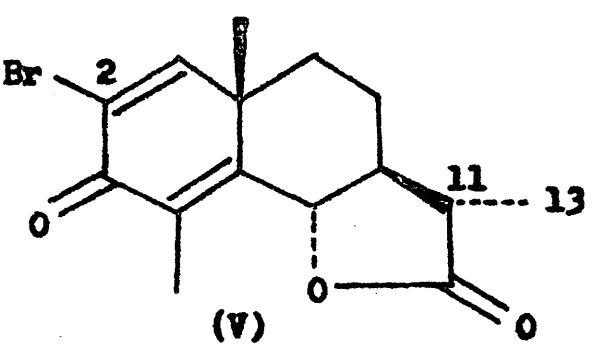
of a C-Br bond about 0.03 Å. The estimated standard deviation of a valency angle is about 3°. Intramolecular bonded distances, intramolecular non-bonded distances <4 Å, intermolecular distances <4 Å and interbond angles are compiled in Tables 27, 28, 29 and 30 respectively. Final anistropic temperature factors, b_{ij}, appear in Table 31. The final calculated and observed structure amplitudes and phase angles for all reflexions observed are listed in Table 32.

The final electron-density distribution over one molecule is shown by means of superimposed contour sections drawn parallel to (100) in Fig. 25, and the corresponding atomic arrangement is shown in Fig. 26.

The arrangement of the molecules in the crystal as viewed in projection along the a axis is shown in Fig. 27.

4. (8) DISCUSSION

The final results of the analysis have established the constitution and stereochemistry (apart from absolute configuration) of 2-bromo-α-santonin to be as shown below (V)



(V)

The stereochemistry is that proposed by Miki (1955) and differs from that proposed by Cocker and McMurray (1960) in the configuration at C(11). The methyl group on the lactone ring at C(11) is firmly established as α.

Huffman (1963) has suggested a possible explanation to account for the incorrect assignation of the stereochemistry at C(11) deduced from reactions in the desmotroposantonins. He has shown from equilibration studies in alkali that α-desmotroposantonin is more stable than the β epimer, in contrast to assumptions made by earlier workers (see Cocker and McMurray, 1960) and suggests that the acid catalysed conversion to the less stable β epimer was the cause of the incorrect assignation of configuration at C(11); conditions of acid catalysed rearrangements from α to β-desmotroposantonin are such that the product precipitates during the course of the reaction, and thus acid catalysed rearrangement is rendered irreversible by low solubility in the acid employed, dilute sulphuric acid.

The cyclohexane ring adopts the chair conformation. Steric interaction occurs between C(14) and O(17) and is reflected in the non-bonded distance of 2.87 Å (Table 29). The distance in a standard Dreiding model is 2.5 Å, and Fig. 25 shows how they have been pushed apart in the plane of the molecule.

In common with bromodihydroisophoto-α-santonic lactone acetate, 2-bromo-α-santonin has a γ-lactone ring. The equation of the mean plane through atoms C(6), C(11), C(12), O(17) and O(18) is $0.749X - 0.632Y + 0.196Z + 3.731 = 0$. Application of the χ^2 test to these atoms gives $\chi^2 = 0.091$. This denotes that there are no significant deviations of the atoms from the plane, and strengthens McPhail's conclusions. The deviations of the atoms, and C(7), from the plane are listed in Table 22. The displacement, 0.61 Å, of C(7) from the plane is significant, showing the ring to have the envelope conformation.

The design of the lactone ring conforms to the pattern established in Chapter 3. (7) with respect to carbon-oxygen single bond lengths, and angles about the sp^2 carbon atom. The bond C(12)-O(17) adjacent to the carbon-oxygen double bond has a length of 1.34 Å, whereas the bond C(6)-O(17) which is not adjacent to the carbon-oxygen double bond has a length of 1.45 Å showing partial double bond character in the C(12)-O(17) bond. The angle C(11)C(12) O(17) is approximately tetrahedral (110.8°) while the average exocyclic angle at C(12) is 125°.

Molecular dimensions are in accordance with normally accepted values. Thus the average carbon (sp^3)-carbon (sp^3) single bond length of 1.55 Å is in favourable agreement with

the value of 1.545 Å in diamond. The average carbon (sp^3)-carbon (sp^2) single bond length of 1.49 Å, agrees well with the value of 1.507 Å predicted by Dewar and Schmeising(1959) and the value of 1.49 Å reported in 5-bromogriseofulvin (Brown and Sim, 1963). The average carbon (sp^2)-carbon (sp^2) single bond length of 1.49 Å is close to the value of 1.479 Å proposed by Dewar and Schmeising (1959) and that of 1.48 Å found in benzoic acid (Sim, Robertson and Goodwin, 1955). The average carbon-carbon double bond length, 1.32 Å, compares favourably with the values of 1.33 Å and 1.32 Å reported for ethylene (Bartell and Bonham, 1957) and para-benzoquinone (Trotter, 1960) respectively.

The average carbon-oxygen double bond length has a value of 1.19 Å which is not significantly shorter than that of 1.21 Å predicted by Pauling (1960) or that of 1.22 Å found in para-benzoquinone (Trotter, 1960). The carbon (sp^2) bromine distance of 1.92 Å is not significantly different from the average value of 1.89 Å reported for various olefinic bromides (Sutton et al., 1958).

In the cyclohexadieneone ring system the average bond angle is 120° which is the value expected for a planar strain free system. The equation of the mean plane C(1), C(2), C(3), C(4), C(5) and C(10) is $0.999X - 0.020Y - 0.010Z - 1.546 = 0$. The displacements of the atoms from the plane are C(1) - 0.006, C(2) + 0.010,

C(3) + 0.001, C(4) - 0.017, C(5) + 0.020 and C(10) - 0.008. Application of the χ^2 test to these six atoms, where $\chi^2 = 0.96$, shows no significant deviation from planarity.

The average bond angle in the cyclohexane ring is 111° which is close to the tetrahedral value. In common with the five-membered rings discussed in Chapter 3.(8) the average bond angle (105°) is smaller than tetrahedral.

The intermolecular contacts (Table 29) are all greater than 3.4 Å and correspond to normal van der Waals interactions; the shortest separations involve oxygen atoms.

PART V.

THE STRUCTURE OF ERGOFLAVIN : X-RAY ANALYSIS OF TETRAMETHYLERGOFLAVIN DI - p - IODOBENZOATE

5. (1) INTRODUCTION

Ergot, the sclerotia produced by the fungus Claviceps purpurea when grown on rye, contains 1-2% of colouring matter (Eglinton, G., King, F.E., Lloyd, G., Loder, J. W., Marshall, J. R., Robertson, A. and Whalley, W. B. (1958)).

In general, the structures of the compounds have not been elucidated. One, ergoflavin, was first isolated by Freeborn (1912) and has been the subject of investigation by Eglinton et al. (1958) who have suggested the molecular formula $C_{30}H_{26}O_{14}$, and the presence of a diphenyl nucleus, four phenolic and two alcoholic hydroxyl groups, two γ-lactone rings and two carbonyl groups. It is further suggested that the molecule is symmetrical, being produced in nature by oxidative coupling of identical C_{15} fragments.

The di-para-iodobenzoate of tetramethyl ergoflavin was prepared by Professor Whalley for the x-ray structure analysis.

5. (2) EXPERIMENTAL

The collection of crystal data followed a similar course to that in the previous structures analysed.

Cell dimensions were measured from precession, oscillation and rotation photographs, and the intensities

of the hk0....7 layers estimated visually from moving film photographs. The 2963 independent intensities were reduced to structure amplitudes in the usual fashion. No absorption corrections were applied.

The space group was assigned from systematically absent spectra, and the crystal density was measured using a flotation technique in an aqueous solution of zinc chloride.

5. (3) CRYSTAL DATA

$C_{48}H_{40}O_{16}I_2$ M = 1127

Orthorhombic

a = 13.23 Å ± 0.03 Å

b = 38.70 Å ± 0.08 Å

c = 9.37 Å ± 0.03 Å

Volume of the unit cell = 4797 $Å^3$

For Z = 4, ρ(calculated) = 1.561 g./ml.

ρ(measured) = 1.539 g./ml.

F(000) = 2248

Absorption coefficient for x-rays (λ = 1.542) = 111.2 $cm.^{-1}$

Systematic absences: (h00) = 2n + 1

(0k0) = 2n + 1

(00ℓ) = 2n + 1

Space group $P2_12_12_1$ - D_2^4.

5. (4) LOCATION OF THE HEAVY ATOMS

The asymmetric unit in tetramethyl ergoflavin di-para-iodobenzoate has two iodine atoms, and two classes of vector are present in the Patterson syntheses. The first class embraces the two sets of vectors arising from each of the symmetrically related heavy atoms (see Chapter 3. (4)) and the second class results from vectors between non-symmetrically related iodine atoms.

In this analysis, the heavy atom coordinates were derived from the Patterson projection down (001), Harker sections $P(UV\frac{1}{2})$, $P(U\frac{1}{2}W)$, and line sections parallel to (001).

In the two-dimensional Patterson map, at $X = \frac{1}{2}$, $Y = 18.46/120 = \frac{1}{2} - 2y_1$ and $y_1 = 20.77/120$; also $Y = 27.76/120 = \frac{1}{2} - 2y_2$, and $y_2 = -16.13/120$. These peaks are marked A and B respectively in Fig. 28. At the peak marked C, $Y = \frac{1}{2}$, $X = 7.63/40 = \sim 2x_1 = \sim 2x_2$ (the two vectors are superimposed) and $x_1 = \sim x_2 = \sim 3.81/40$. At the general peak marked D, $U = \frac{1}{2} - 2x_1$, $V = 2y_1$ whence $x_1 = 4.03/40$ and $y_1 = 20.74/120$, and at E, $U = \frac{1}{2} - 2x_2$, $V = -2y_2$ whence $x_2 = 3.12/40$ and $y_2 = -16.17/120$.

The vectors between I_1 and I_2 are marked F, G, H and J. At F, $U = x_1 - x_2$ and $V = y_1 - y_2$, at G, $U = -\frac{1}{2} + x_1 + x_2$ and $V = y_1 + y_2$, at H, $U = x_1 + x_2$ and $V = -\frac{1}{2} + y_1 - y_2$, and at J, $U = -\frac{1}{2} + x_1 - x_2$ and $V = -\frac{1}{2} + y_1 + y_2$.

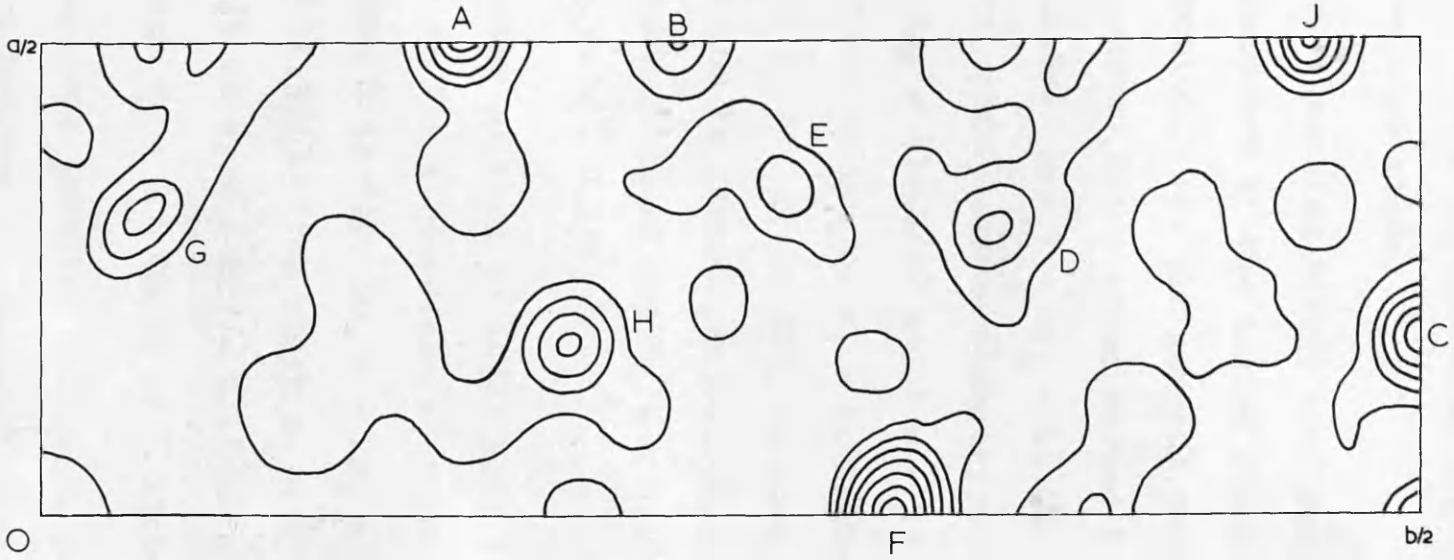


Fig. 28. Tetramethylriboflavin di-para-iodobenzoate.
(OOk) Patterson projection. Contours are at equal and arbitrary intervals. The I-I vectors discussed in the text are marked A, B, C, D, E, F, G, H, and J.

The values of x_1, y_1, x_2 and y_2 reduced from these vectors concord with the values derived from the vectors between symmetrically related atoms.

The Harker section $P(UV\frac{1}{2})$ confirmed the values of the x and y parameters of the iodine atoms deduced from the Patterson projection. At the general peak between symmetrically related heavy atoms marked K in Fig. 29, $U = \frac{1}{2} - 2x_1 = 11.9/40$ and $V = 2y_1 = 41.62/120$ while at L, the other general peak between symmetrically related iodine atoms, $U = \frac{1}{2} - 2x_2 = 13.44/40$ and $V = -2y_2 = 32.26/120$. These vectors yield values of $x_1 = 4.01/40$, $y_1 = 20.81/120$, $x_2 = 3.23/40$ and $y_2 = -16.13/120$, in good agreement with the values from the Patterson projection. The averages of the several values obtained are $x_1/a = 0.101$, $y_1/b = 0.173$, $x_2/a = 0.079$, $y_2/b = -0.135$.

The Harker section at $(U\frac{1}{2}W)$ gave four alternative values for each of the z coordinates of the iodine atoms. At the peak marked M in Fig. 30, $U = 2x_1 = 8.24/40$ and $W = \pm\frac{1}{2} \pm 2z_1 = 11.95/30$ from which $z_1 = \pm 1.52/30$ or $\pm 13.48/30$, while at N, $U = 2x_2 = 6.40/40$ and $W = \pm\frac{1}{2} \pm 2z_2 = 5.11/30$ from which $z_2 = \pm 4.94/30$ or $\pm 10.06/30$.

Line sections parallel to W were computed through the following vectors between non-symmetrically related iodine atoms:

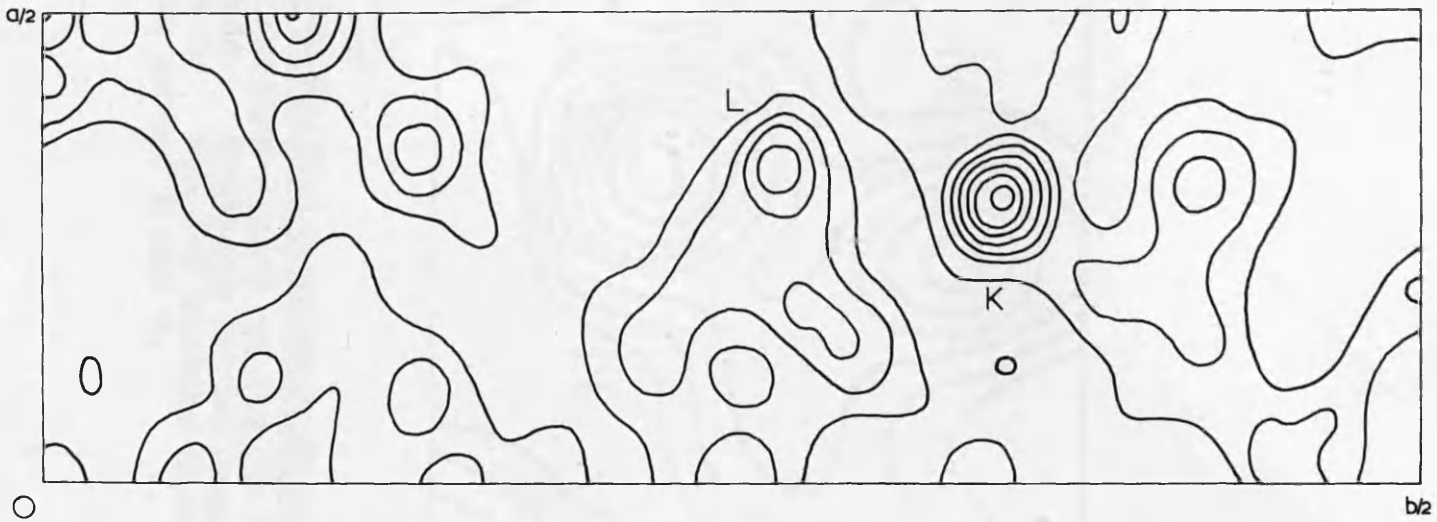


Fig. 29. Tetramethylriboflavin di-para-iodobenzoate.

Harker section P(UV½) of the 3D Patterson synthesis. Contours are at equal and arbitrary intervals. The I-I vectors discussed in the text are marked K and L.

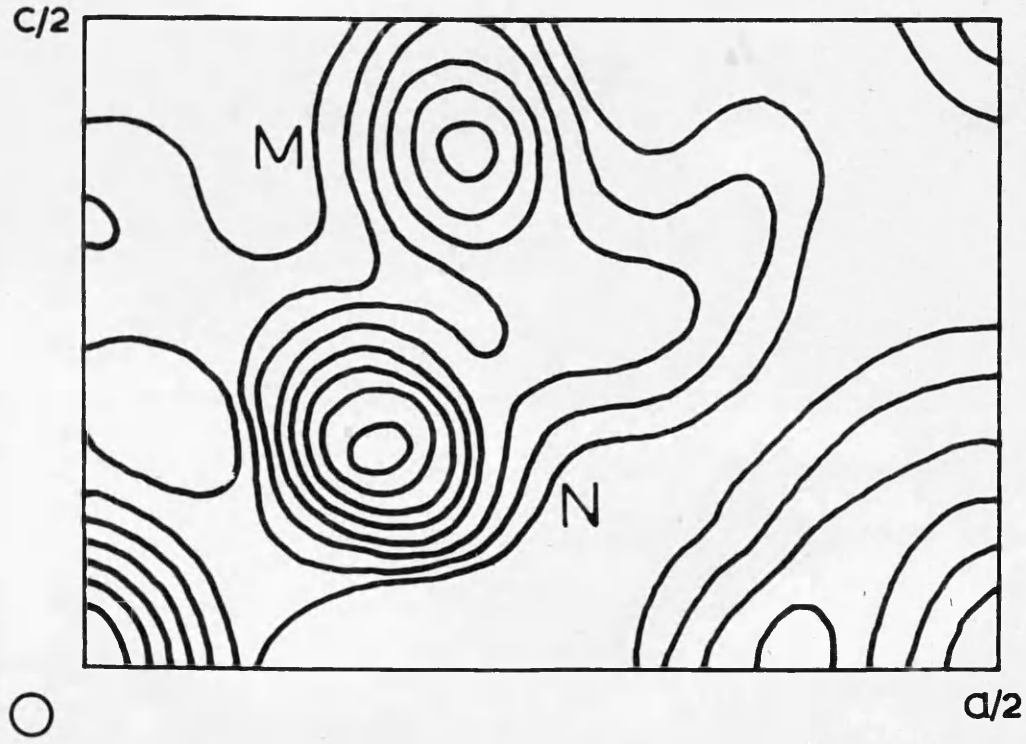


Fig. 30. Tetramethylacriflavin di-para-iodobenzoate.

Harker section P(U½w) of the 3D Patterson synthesis. Contours are at equal and arbitrary intervals. The I-I vectors discussed in the text are marked M and N.

$x_1 - x_2, y_1 - y_2, z_1 - z_2$; $-\frac{1}{2} + x_1 - x_2, -\frac{1}{2} + y_1 + y_2, z_1 + z_2$; $-\frac{1}{2} + x_1 + x_2, y_1 + y_2, -\frac{1}{2} + z_1 - z_2$; $x_1 + x_2, -\frac{1}{2} + y_1 - y_2, -\frac{1}{2} + z_1 + z_2$. Maxima occurred at W = 68/240, 93/240, 52/240 and 27/240 respectively. These values are consistent with values of 1.52/30 for z_1 and 10.06/30 for z_2.

Thus an unambiguous solution of the vector maps was accomplished. The coordinates of the heavy atoms are:

I_1	x/a = 0.101	y/b = 0.173	z/c = 0.051
I_2	x/a = 0.079	y/b = - 0.135	z/c = 0.335

5. (4) SOLUTION OF THE STRUCTURE

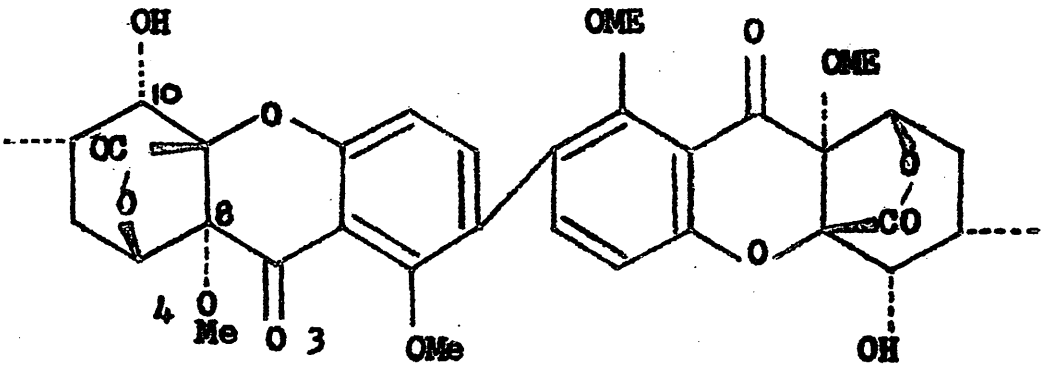
The structure was solved directly from the first three dimensional Fourier synthesis which was based on the phases of the iodine atoms. Assuming a temperature factor of $B_\theta = 3.5$ Å^2, the overall discrepancy of the structure factors was 33%, and all the atoms of the structure were clearly resolved.

In a second structure factor calculation, which included all the light atoms (except hydrogen) as carbon atoms, the discrepancy fell to 23.1%, and the resulting three-dimensional Fourier map showed very little spurious electron density.

A third structure factor calculation in which the atoms were assigned their correct chemical type, reduced the

R value to 20.7%. The superimposed contour sections of the second electron-density map, drawn parallel to (001) is shown in Fig. 31.

The results have established the structure and relative stereochemistry of tetramethyl ergoflavin di-para-iodobenzoate and it follows, that the structure and stereochemistry of ergoflavin is as in (VI)



(VI)

The absolute stereochemistry has been established both chemically, by oxidation of ergoflavin to (-)-methyl succinnic acid (Whalley, 1963) and by x-ray methods, employing the anomolous dispersion technique of Bijvoet (McPhail, 1963).

Ergoflavin (VI) consists of two identical fragments, each having three six-membered and a five-membered ring. Each fragment consists of a γ-pyrone ring flanked on one side by a benzene ring, and on the other side by a cyclohexane ring

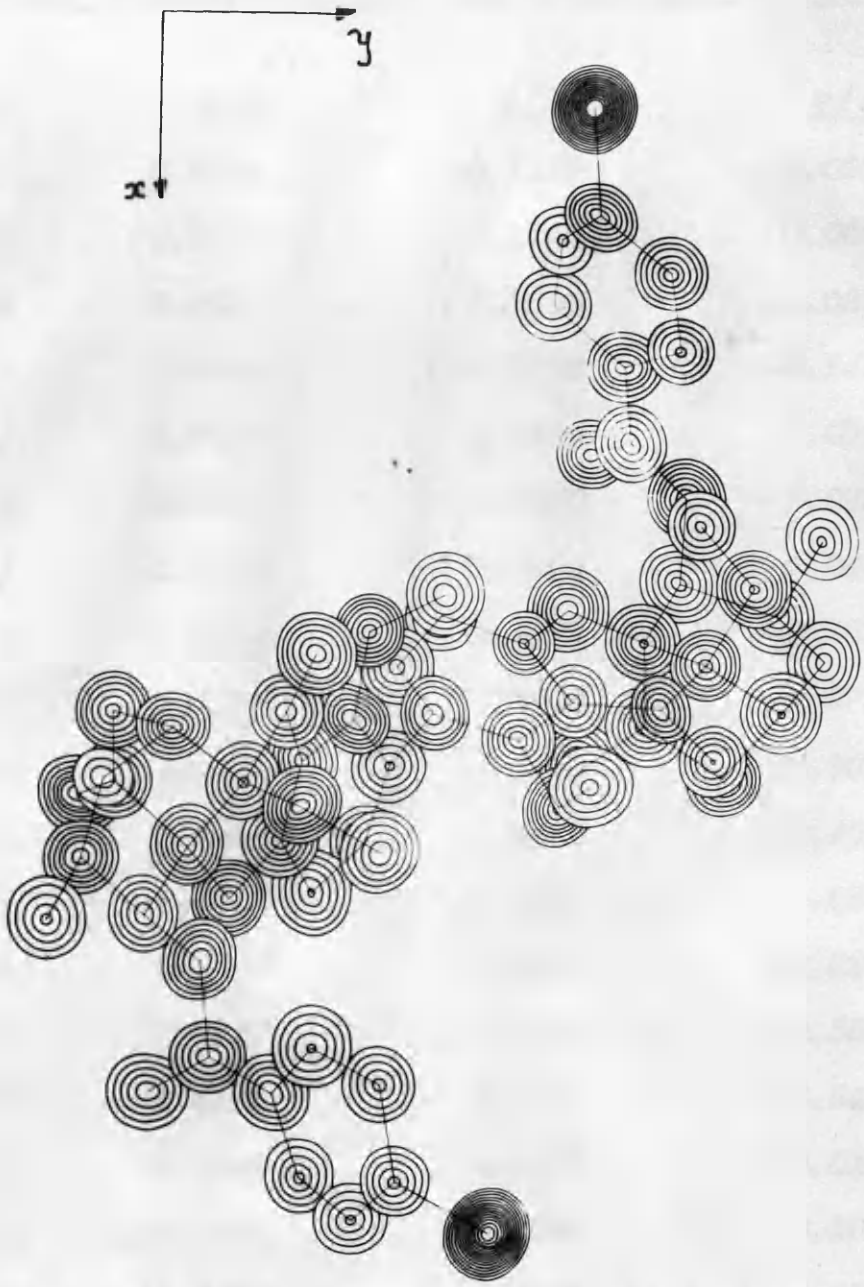


Fig.1. Interpretation of Patterson of di-para-iodobenzoate.

Superimposed contoured sections of the Pat 3D Fourier synthesis covering the region of one molecule. The sections are drawn at intervals of 1 electron/Å^3 omitting the 1 electron contour. The interval for the iodine atoms is 3 electrons/Å^3.

TABLE 33.

Tetramethyl Ergoflavin Di -p- iodobenzoate

Atomic Coordinates from the Second 3D Fourier Synthesis

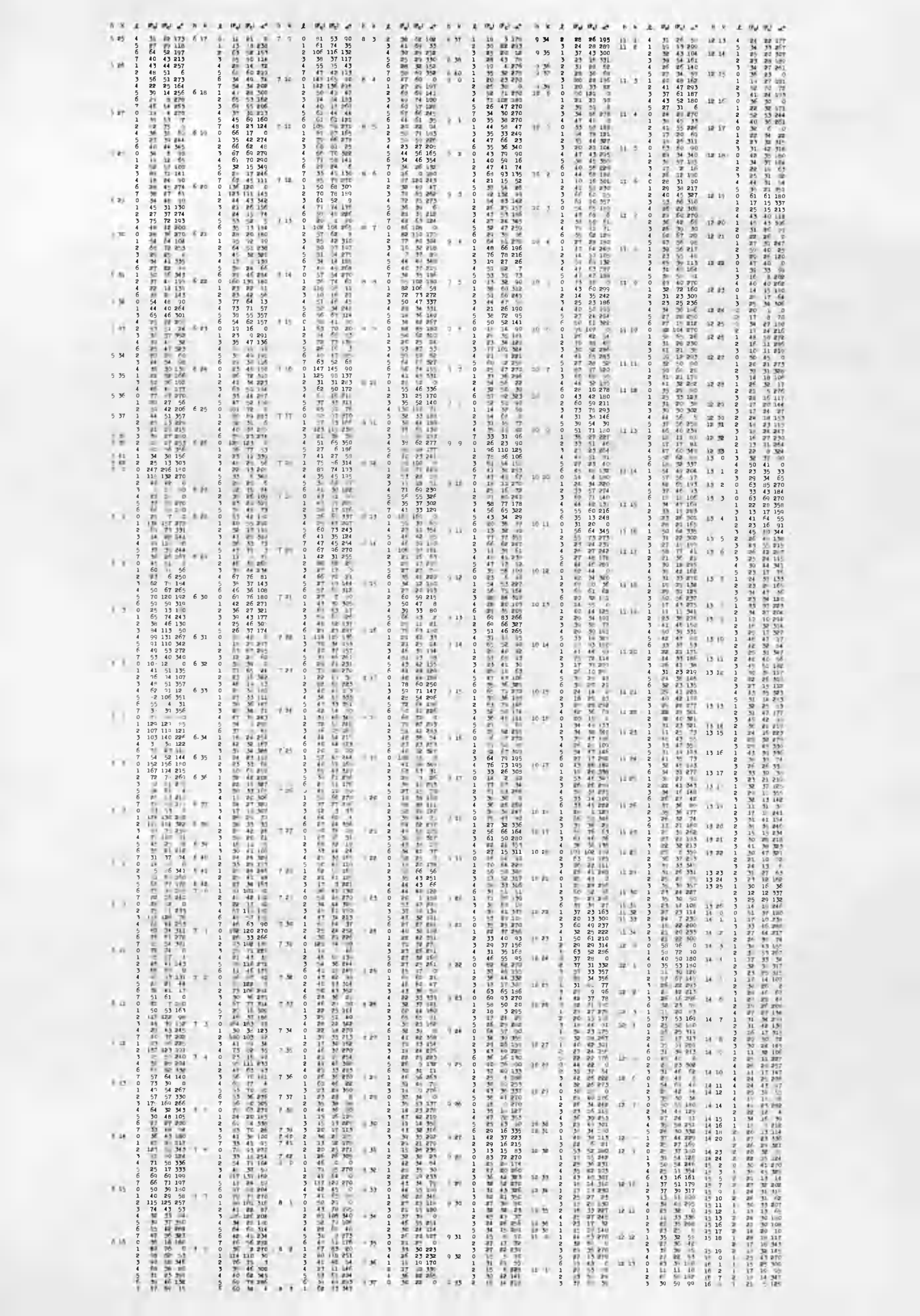
Atom	x/a	y/b	z/c
C(1)	0.8136	0.1122	0.0981
C(2)	0.8464	0.1443	0.0691
C(3)	0.8010	0.1663	-0.0375
C(4)	0.7345	0.1474	-0.1411
C(5)	0.7027	0.1149	-0.0992
C(6)	0.7651	0.0970	0.0227
C(7)	0.8017	0.2028	-0.0429
C(8)	0.7570	0.2189	-0.1612
C(9)	0.7314	0.1945	-0.2777
C(10)	0.6621	0.2082	-0.4077
C(11)	0.6987	0.2473	-0.4553
C(12)	0.7501	0.2661	-0.3535
C(13)	0.8123	0.2491	-0.2378
C(14)	0.6133	0.2649	-0.5521
C(15)	0.8293	0.1948	-0.3426
C(16)	0.5969	0.2175	-0.0114
C(17)	0.9024	0.1737	0.3017
C(18)	0.4986	0.1894	-0.4179
C(19)	0.4055	0.1864	-0.3301
C(20)	0.3410	0.1578	-0.3610
C(21)	0.2558	0.1595	-0.3232

Atom	x/a	y/b	z/c
C(22)	0.2287	0.1760	-0.1852
C(23)	0.2988	0.2046	-0.1579
C(24)	0.3925	0.2086	-0.2339
C(1)'	0.8777	0.0935	0.2182
C(2)'	0.8281	0.0799	0.3323
C(3)'	0.8714	0.0597	0.4444
C(4)'	0.9662	0.0508	0.4161
C(5)'	1.0242	0.0645	0.3017
C(6)'	0.9662	0.0842	0.1910
C(7)'	0.8163	0.0529	0.5686
C(8)'	0.8991	0.0364	0.6951
C(9)'	0.9757	0.0153	0.6268
C(10)'	1.0510	-0.0013	0.7287
C(11)'	0.9903	-0.0263	0.8182
C(12)'	0.8988	-0.0184	0.7536
C(13)'	0.8350	0.0085	0.7628
C(14)'	1.0605	-0.0406	0.9314
C(15)'	0.9000	-0.0153	0.5421
C(16)'	0.9786	0.0909	0.7593
C(17)'	0.6781	0.1156	0.3923
C(18)'	1.2168	0.0240	0.7666
C(19)'	1.2577	0.0494	0.8711
C(20)'	1.3522	0.0604	0.8447

Atom	x/a	y/b	z/c
C(21)'	1.4058	0.0811	0.9235
C(22)'	1.3578	0.0988	1.0166
C(23)'	1.2488	0.0911	1.0469
C(24)'	1.2030	0.0649	0.9550
O(1)	0.9244	0.1612	0.1560
O(2)	0.6930	0.1640	-0.2590
O(3)	0.8680	0.2228	0.0300
O(4)	0.6665	0.2389	-0.1152
O(5)	0.8900	0.2255	-0.3216
O(6)	0.8789	0.1686	-0.4039
O(7)	0.5653	0.2110	-0.3510
O(8)	0.5142	0.1735	-0.5417
O(1)'	0.7244	0.0853	0.3314
O(2)'	1.0288	0.0322	0.5095
O(3)'	0.7490	0.0653	0.6166
O(4)'	0.9292	0.0596	0.7982
O(5)'	0.8198	-0.0159	0.6521
O(6)'	0.9164	-0.0277	0.4417
O(7)'	0.1120	0.0207	0.7892
O(8)'	1.2606	0.0012	0.6888
I	0.1006	0.1734	-0.0508
I'	1.4211	0.1347	1.1681

Table 34. Tetramethylergoflavin di-para-iodobenzoate.

Measured and calculated values of the third set structure factors.



bridged at C(9) and C(13) by a γ-lactone system: the benzene rings are joined to form the diphenyl nucleus.

Two phenolic and four alcoholic hydroxyl groups are present in place of the four phenolic and two alcoholic hydroxyl groups expected. The phenolic character of the alcoholic OH groups may be accounted for by steric interaction between the alcoholic hydroxy groups at C(8) and C(10) and perhaps hydrogen bonding involving O(3) and O(4).

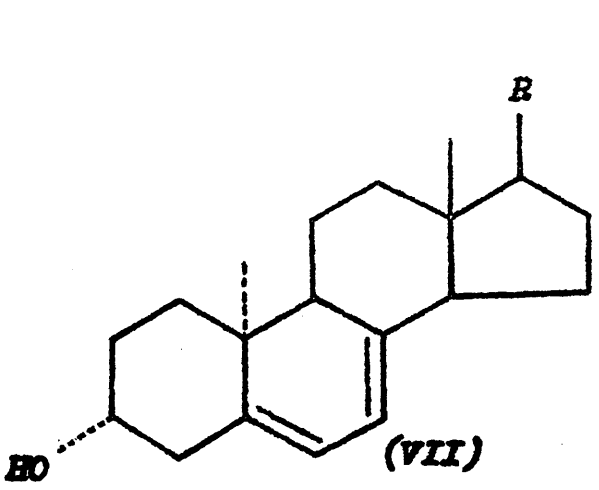
Refinement of the structure is now in progress.

PART VI.

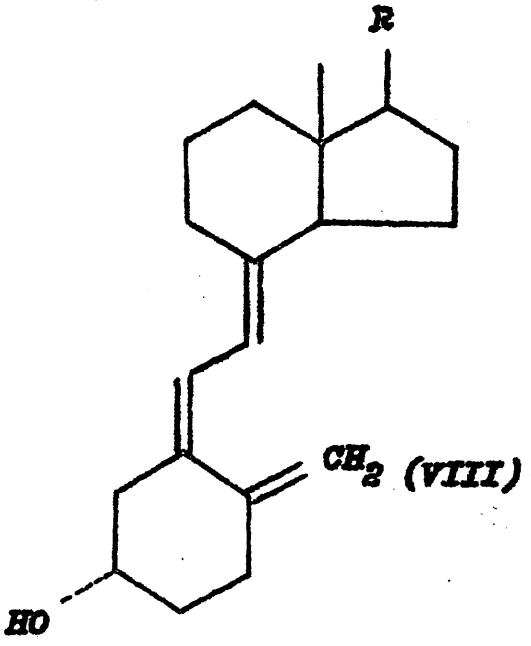
TOXISTEROL A_2 : X-RAY STUDY OF THE 4 - IODO - 3 - NITRO-BENZOATE

6. (1) INTRODUCTION

Irradiation of ergosterol (VII) with ultraviolet light gives the fat-soluble vitamin D_2 or calciferol (VIII); the structures and chemistry of the intermediates are well characterised (Fieser and Fieser, 1959).



(VII)



(VIII)

One of the products of over-irradiation of ergosterol is toxisterol A_2 whose constitution is entirely unknown. A further product of over-irradiation is suprasterol II which has been assigned an interesting spirocyclic structure both by chemical methods (Dauben, and Baumann, 1961) and by an x-ray analysis of the 4-iodo-5-nitrobenzoate (Saunderson and Hodgkin 1961).

X-ray methods have also been employed in the elucidation of the structure of calciferol (Crowfoot Hodgkin,

Webster and Dunitz, 1957) and lumisterol, the 10 epimer of ergosterol (Crowfoot Hodgkin and Sayre, 1952), and due to the small amount of toxisterol A_2 available, an x-ray study of the 4-iodo-3-nitrobenzoate derivative, prepared by Professor Barton, was undertaken.

Details of the crystal data were investigated during the course of his B.Sc. thesis work by A. C. MacDonald (1962).

6. (2) EXPERIMENTAL

Unit cell data were measured from precession photographs, and the space group determinated from systematically absent spectra; $P2_1/m$ must be rejected because the compound is optically active. The density of the crystal was found by a flotation technique in aqueous potassium iodide solution. The molecular weight of toxisterol A_2 has not been determined, and the value of 396, the molecular weight of ergosterol, calciferol and suprasterol II was assumed.

Small crystals were used for intensity data collection, and no absorption corrections were made. The $0k\ell$ ... $4 \pm k\ell$ data were estimated in the usual visual fashion from Weissenberg photographs. Initial difficulty in indexing the reflections was overcome by comparison with

$h0\ell$ and $hk0$ precession photographs.

Structure amplitudes were obtained from intensities using the mosaic crystal formula, the normal corrections for Lorentz, polarization, and rotation factors for upper layers being made. The exposure times for the individual layers were approximately similar, and it was assumed that the layers were all on the same relative scale.

In all, 1436 independent structure amplitudes were measured, of which 157 were smaller than the least observable value.

6. (3) CRYSTAL DATA

$C_{35}H_{46}O_4$ NI

Monoclinic, c unique

$M = 671.7$

$a = 6.38\ \text{Å} \pm 0.02\ \text{Å}$

$b = 11.35\ \text{Å} \pm 0.02\ \text{Å}$

$c = 24.36\ \text{Å} \pm 0.02\ \text{Å}$

$\gamma = 105^\circ 10'$

Volume of the unit cell $= 1706.3\ \text{Å}^3$

For $Z = 2$, ρ(calculated) $= 1.308$ g./ml.

ρ(measured) $= 1.300$ g./ml.

$F(000) = 696$

Absorption coefficient for x-rays (λ=1.542) $= 78.1\ \text{cm.}^{-1}$

Systematic absences: $(00\ell) = 2n + 1$

Space group $P2_1 - C_2^2$.

6. (4) LOCATION OF THE HEAVY ATOM

The expression for the Patterson function for a crystal belonging to the monoclinic system with C unique is

$$P(UVW) = \frac{4}{Vc^2} \sum_0^\infty \sum_0^\infty \sum_0^\infty \left[|F(hk\ell)|^2 \cos 2\pi (hU + kV) + |F(h\bar{k}\ell)|^2 \cos 2\pi (-hU + kV) \right] \cos 2\pi \ell W$$

and this is simply reduced to the corresponding two-dimensional expression for projections.

The equivalent positions in the space group $P2_1$ are x, y, z and $\bar{x}, \bar{y}, \frac{1}{2} + z$ and consequently the iodine-iodine vector occurs at $U = 2x$, $V = 2y$, $W = \frac{1}{2}$. The z parameter of the iodine atom in the structure may be assigned arbitrarily.

The y coordinate of the iodine atom was found from the two-dimensional Patterson synthesis projected along (100) using 197 coefficients. The peak marked A in Fig. 32 indicates clearly the vector expected at $V = 2y$, $W = \frac{1}{2}$ and leads to a value for the iodine atom of $y = \sim 0$, although the elongation of the peak along V indicates that the y coordinate should exceed zero slightly.

The Harker section of the full three-dimensional Patterson synthesis at $W = \frac{1}{2}$ showed one outstanding peak marked B in Fig. 33, and this corresponds with the vector $U = 2x$, $V = 2y$ and verifies the value assigned to the y

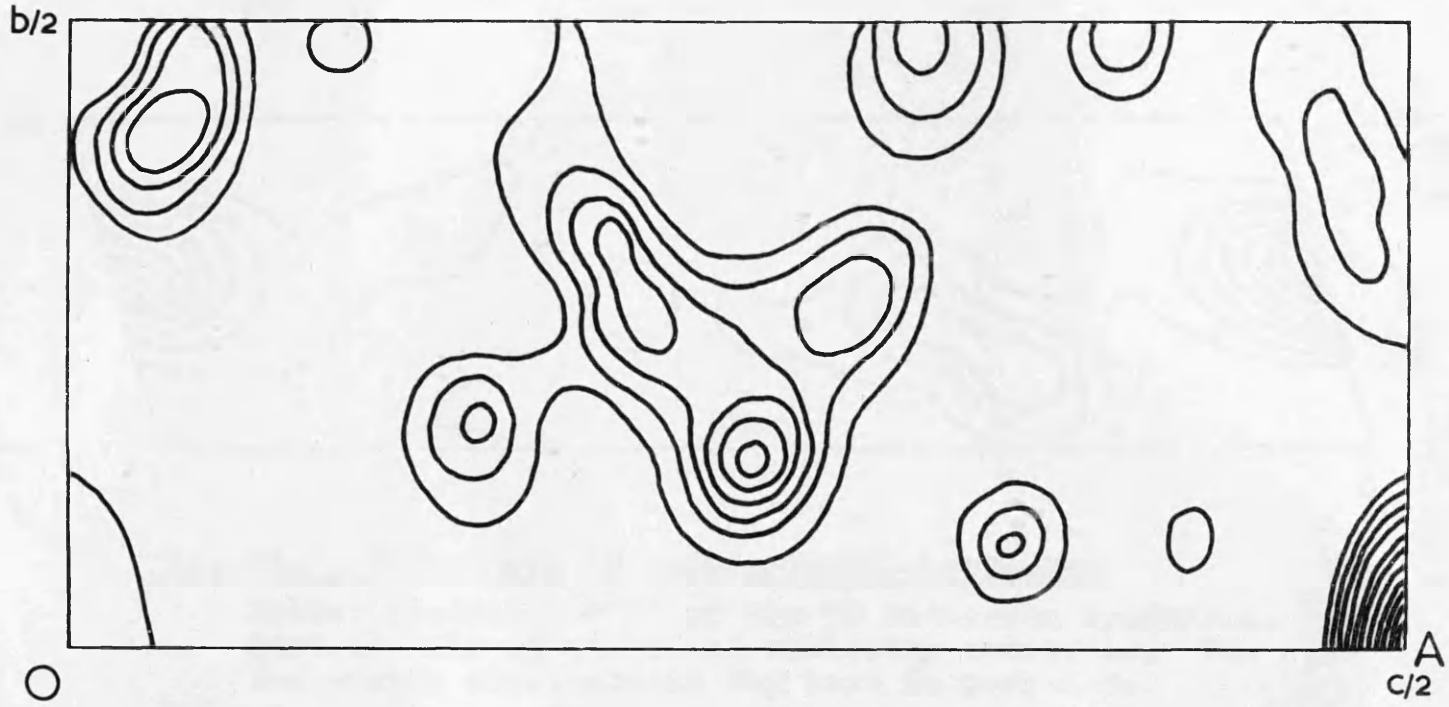


Fig. 32. Toxisterol A_2 4-iodo-3-nitrobenzoate.

(100) Patterson projection. Contours are at equal and arbitrary intervals. The I-I vector discussed in the text is marked A.

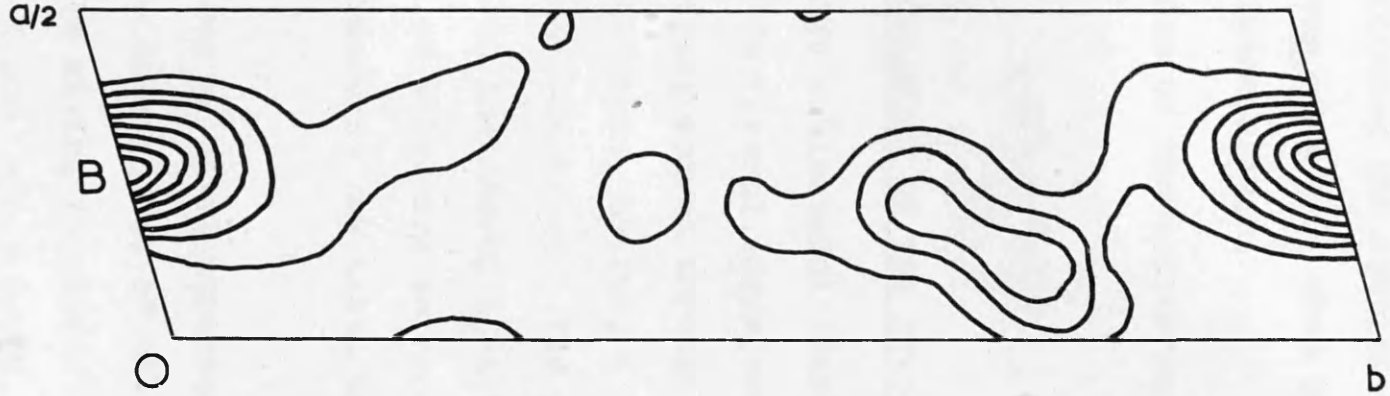


Fig. 33. Toxisterol Ab 4-iodo-3-nitrobenzoate.

Harker section P(UV½) of the 3D Patterson synthesis. Contours are at equal and arbitrary intervals. The I-I vector discussed in the text is marked B.

coordinate from the Patterson projection. The value of the x coordinate is 0.125. Confirmation of these values was obtained by computing the section of the three-dimensional Patterson at U = $\frac{1}{4}$, when the expected vector at V= 0 and W = $\frac{1}{2}$ materialised.

The solution of the vector maps is consistent with iodine coordinates of

x/a = 0.125 y/b = 0.010 z/c = 0.250

6. (5) ATTEMPTED SOLUTION OF THE STRUCTURE

The steroids cholesterol (Carlisle and Crowfoot Hodgkin, 1945) and lumisterol (Crowfoot Hodgkin and Sayre, 1952) were successfully solved through the centrosymmetric projection about the short, unique, b axis (space group $P2_1$) using the heavy atom technique. The projection down the unique, 24 Å, axis in toxisterol A_2-4-iodo-3-nitrobenzoate would be difficult to solve due to superimposition of atomic sites, and recourse was taken to three-dimensional methods.

Several sets of trial structures were computed varying the x and y coordinates of the iodine atom. The iodine atom position giving the lowest discrepancy was x/a = 0.133, y/b = 0.000, z/c = 0.250. F_c equalled zero when h = 0, l = 2n + 1, and due to the proximity of x/a

to the value of 0.125, Fc approached zero when $h = 2$, $\ell = 2n$, and $h = 4$, $\ell = 2n + 1$. Thus the discrepancies for layers $h = 0, 1 \ldots, 4$, were 65%, 43%, 56%, 46% and 50% respectively. The trend in magnitude of the structure factors paralleled exactly that in the intensities, substantiating the correctness of indexing of the latter.

The phase angles from the iodine contribution were 0 and π, and consequently the first three-dimensional electron density map had a spurious centre of symmetry. In addition to any real atom at P, which occurred at the positions (1) x, y, z; (2) $\bar{x}$, $\bar{y}$, $\frac{1}{2} + z$ in the unit cell, the calculated pattern showed evidence of additional unreal atoms (3) $\bar{x}$, $\bar{y}$, $\bar{x}$; (4) x, y, $\frac{1}{2}-z$ produced by the operation of the centre of symmetry. Of these, atoms (1) and (4), and (2) and (3) are related to one another by mirror planes of symmetry at $z = \frac{1}{4}$ and $\frac{3}{4}$.

Concentrations of peaks on the mirror planes were prominent, but the only structural feature which could be resolved from the map was a six membered cyclohexane ring. Several large peaks near the iodine atom were present, but none defined the position of the benzene ring.

The six atoms forming the cyclohexane ring were included in a second round of structure factor calculation. The discrepancy fell by three per cent, although it was

realized that R would probably drop even if atoms were wrongly placed because so many reflections calculated zero or approximately zero when the calculations were based on the iodine atom alone.

Pseudo-symmetry persisted in the resulting electron-density map as did the concentrations of peaks on the mirror planes. The section at $z = \frac{1}{4}$, drawn up to give a clearer view of this electron density concentration, yielded no information.

Since no progress was made from the second Fourier synthesis, an attempt was made to gauge the validity of possible atomic sites. Several sets of structure factors for the h = 1 layer were calculated: iodine and the cyclohexane ring; iodine, with the ring and its mirror image both at half weight; iodine, with the eight largest peaks and mirror images all at half weight; iodine and a very unlikely benzene ring on the mirror plane; and iodine with the two largest peaks on the mirror plane. In all cases the discrepancy rose.

A two-dimensional Fourier synthesis projected down the short axis was computed. The trend of electron density indicated that the only likely orientation of the benzene ring was in the a b plane, but previous attempts to locate the ring in this plane from the three-dimensional Fourier

synthesis were unsuccessful.

Attention was next focussed on an attempt to locate light atoms from the three-dimensional Patterson synthesis using superposition methods. The method used was essentially the one employed in the structural determination of strychnine hydrogen bromide (Robertson and Beevers, 1951). The Patterson function was set down with its origin at each of the known atomic positions of the iodine atom, so that the vectors of each of the lighter atoms with the heavy atoms were brought into coincidence on atomic sites. Where overlap occurred, the validity of the possible atomic sites was determined by the minimum value of the Patterson function at the point of concurrence. The first three-dimensional Fourier map was also superimposed on the Patterson functions and the positions of coincident peaks marked.

Thirty six possible atomic sites were located and these were arranged in nine groups in order of estimated correctness. Structure factors of each of the nine groups in turn were calculated over the 360 reflections for which $h = 1$. The discrepancy rose in each case.

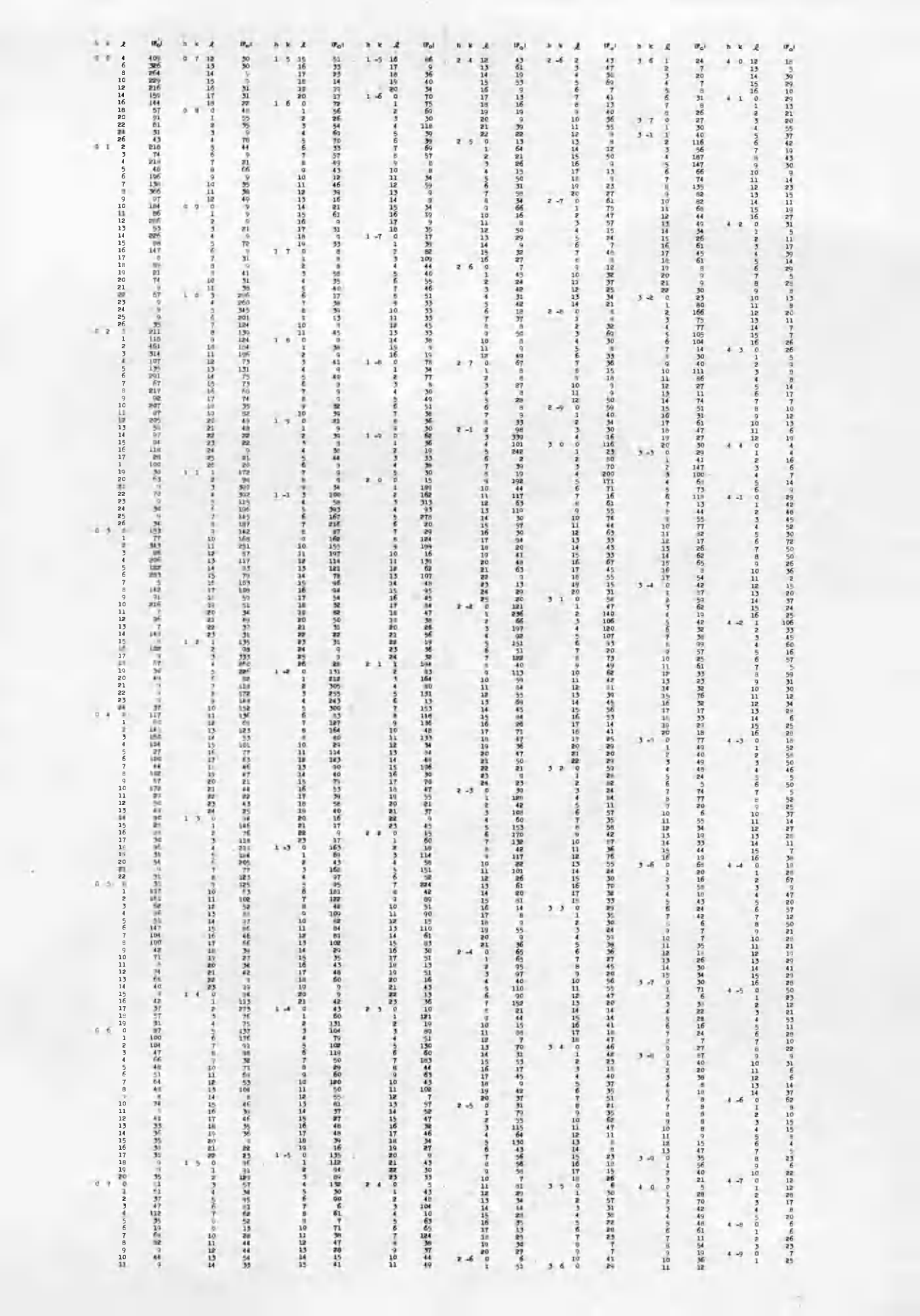
In trying to account for the failure of the superposition method where knowledge of phases is not necessary and all the data is employed, several factors must be taken into account. Firstly, the presence of iodine

atoms results in considerable absorption. Secondly, the data fades out at low $\sin\theta$ values, and thirdly, there are nearly 1000 interatomic vectors in the unit cell excluding hydrogen atoms. A large measure of coincidence is certain and resolution must be restricted. Consequently it is probable that many atomic sites were inaccurately or wrongly assigned.

The likeliest route to solving the structure appears to lie in the preparation of a further derivative in which the heavy atom assumes a general position, and the space group has no ambiguous symmetry.

Table 35. Toxisterol A$_2$ 4-iodo-3-nitrobenzoate.

Observed values of the structure amplitudes.
The values are on the same relative scale.



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